Thermo Scientific
**Protein Biology Products
for Neurobiology Research**

get insights

reagent tools for revolutionizing neurobiology research

- neuronal protein extraction kits • GTPase research tools
- tandem mass tag® technology • high content analysis • antibodies

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Neuroscience Technical Handbook

reagent tools for revolutionizing neurobiology research

An overview

Neurobiology has rapidly become one of the most important and exciting areas of life science research. The field of neurobiology involves studying how cells of the nervous system process information and mediate behavioral changes. The nervous system is composed of neurons and other supportive cells, such as glial. These cells compose the functional circuits that sense and respond to biological signals. Understanding the molecular mechanisms of nerve function continues to be a major focus of neuroscience research, including how neurotransmitters and electrical signals are processed by neurons (e.g., dendrites, axons). Neuronal differentiation, growth, survival and regeneration are also key areas of investigation. Neurobiology research has far reaching implications into human health, including development, memory, mood disorders, aging and disease.

The increasing rate of occurrence of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, have highlighted the need to better understand the cellular mechanisms and pathways involved in neural function. A common theme is that accumulation of insoluble filamentous aggregates of normally soluble proteins characterizes nearly every major degenerative disease. Amyloid-b (Ab) peptide and Tau are two proteins at center stage of this research.

Disruption of the regulation by key neural proteins has enabled researchers to identify hallmark events in the progression of neurodegenerative diseases. For example, changes in Tau phosphorylation and the ratio of splice variants that compose Tau aggregates have provided insight to its role in Alzheimer's and Parkinson's diseases. Mapping the cleavage pathways of amyloid precursor protein (APP) using different secretases has increased understanding of b-amyloid peptide formation and amyloidogenesis. Detection of gg enolase (enolase 2) in cerebral spinal fluid has become a useful marker for neural damage after injury, stroke and the presence of tumors.

Neurobiology research has been hindered by serious technical limitations. For example, primary neuronal cells were difficult to culture in vitro, traditional transfection reagents were ineffective and toxic for primary neurons, and neurite outgrowth measurements were laborious and time-consuming. Powerful new techniques have been developed to advance the understanding of how the nervous system functions. These include RNA interference (RNAi) for modulating expression levels of key neurobiology targets, high-efficiency transfection reagents for neuronal cells, novel stem cell reagents, optimized neurite outgrowth assays and new antibodies specific for neurobiology and signal transduction pathway studies. This brochure highlights our key technology platforms for revolutionizing neurobiology research and demonstrates our commitment to offering a strong collection of neurobiology research tools and reagents.

products and services to understand neuronal

1 New reagents to extract and protect neuronal proteins

Neuronal tissue and primary cells are frequently used to study proteins involved in learning, behavior and neurodegenerative disease. The morphology and unique lipid composition of the neuronal cell membrane often make protein extraction from all cellular compartments inefficient. Dendrites branch near the neuronal cell body and act as signaling sensors, while the axon extends away from the cell body and transduces signals to the synapse. The insulative myelin sheath that coats axons is rich in glycolipid, sphingomyelin and cholesterol. The use of denaturing detergents overcomes this issue; however, their presence typically compromises protein function. With the introduction of the Thermo Scientific N-PER Neuronal Protein Extraction Reagent, a method now exists to easily isolate active neuronal proteins (**see page 6**).

While many neuroscientists study intact neurons, a significant number focus their efforts on understanding the signal transmission events that occur at the neuronal cell-cell junctions, or synapses. Synaptic proteins can be enriched from synaptosomes, which are isolated nerve terminals generated during the homogenization of nerve tissue. Synaptosomes contain the complete presynaptic terminal, including mitochondria and synaptic vesicles, along with the postsynaptic membrane and the postsynaptic density (Figure 1). They are commonly used to study synaptic function because they contain functional ion channels, receptors, enzymes and proteins, as well as the intact molecular machinery for the release, uptake and storage of neurotransmitters. Preparation of synaptosomes typically involves several centrifugation steps to separate nerve termini from cell bodies and axons. Although synaptosomes are relatively easy to prepare in the laboratory, there has been no commercially available reagent for their efficient and consistent isolation until now. The proprietary Thermo Scientific Syn-PER Synaptic Protein Extraction Reagent efficiently isolates functional synaptosomes containing active synaptic proteins from neuronal tissue and primary cultured neurons (**see page 8**).
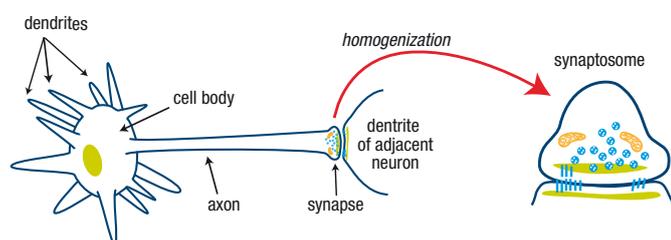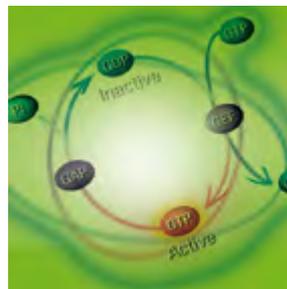
2 GTPases and kinases in neurology

The differentiation of neuronal cells requires signaling pathways that are responsive to the extracellular matrix as well as extensive remodeling of the cytoskeleton. GTPases are critical for these processes. The Ras GTPase family acts as membrane-associated signal transducers; the Rho GTPase family regulates actin and microtubule dynamics. The cellular location 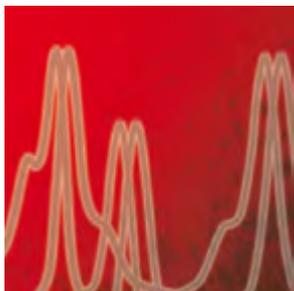of the GTPases and their respective effector binding proteins contribute to cellular differentiation. Thermo Scientific Active GTPase Pull-down and Detection Kits enable detection of active GTPase activity and are an effective tool for studying cellular localization of active GTPase activity (**see page 10**). An application note in which active GTPases were detected and localized during the neuronal cell differentiation process can be found on **page 13**.

Neuroblastoma is a cancerous tumor of nerve tissue that develops in children and is the most common tumor found in children younger than one year of age. Although much genetic data has been collected on these tumors, the biology responsible for this disease is not well understood. Current evidence suggests that the tropomyosin-receptor kinase (Trk) family of neurotrophin receptors plays a critical role in neuroblastoma phenotypes and prognosis. Thermo Scientific Pierce Kinase Enrichment Kits with ActivX* ATP or ADP Probes, found on **pages 16-18**, enable one to study the Trk family in neuroblastoma.

Assessment of active-site labeling can be accomplished by either Western blot or mass spectrometry (MS). Both workflows can be used for determining inhibitor target binding, but only the MS workflow can identify global inhibitor targets and off-targets.

Figure 1. Basic structure of a neuron and a synaptosome containing detached nerve terminal with part of the postsynaptic membrane.

biology and drive knowledge

3 Mass Spectrometry and Neuropeptidomics

The nervous system development, function and viability are maintained via a complex interacting network of signaling pathways and cellular interactions that can be disturbed in response to a number of cellular stresses. Changes to these signaling pathways can have significant consequences to the nervous system. Furthermore, characterizing the myriad of activities that support these complex processes is a daunting task. Improvements in mass spectrometry instrumentation, development of robust bioinformatic software, and new proteomic sample preparation techniques are providing sensitive, efficient technologies. These improvements have enabled researchers to perform large-scale quantitation to identify protein expression, post-translation modifications, subcellular fractionations, protein-protein interactions and protein functions. Tandem Mass Tag* (TMT*) Technology (**see page 23**), Active Site Probes and extraction reagent for neuronal tissue and primary cultured neurons are just some of the new MS tools that facilitate the understanding of how cellular proteomes are regulated in the nervous system in health and disease.

A more recent and fast growing segment of neurobiology is the study of neuropeptides. Neuropeptidomics is the study or detailed analysis of endogenous peptides from the brain. Reproduction, anxiety, stress, pain and growth are some physiological processes that involve neuropeptides. Neuropeptide levels in the brain are low compared to the levels of protein degradation fragments and can hamper neuropeptide studies. For this reason, most neuropeptidomic studies use MS methods to detect and identify peptides of interest, which provides useful information of post-translationally modified forms of peptides. Combining sensitive MS instruments and Thermo Scientific TMT Isobaric Mass Tags for quantitation and characterization provide the specificity and sensitivity needed for quantitative peptidomics. Tools like the Tandem Mass Tag Kits used on normal vs. disease tissues enables identification of differential expression patterns that could lead to biomarkers. **See page 23** for an article on and ordering information for TMT Technology.

4 High-content screening and antibodies for neuronal biology studies

Over the last decade, Imaging Cytometry, also known as High-Content Screening (HCS) or High-Content Analysis (HCA), a proven technology combining automated fluorescence microscopy with multi-parameter quantitative image analysis, has emerged as a powerful method for the large-scale study of cell biology. High-content technology provides researchers with the tools to rapidly gain insightful knowledge about targets or compounds of interest in the context of the cell, allowing for better decision-making and increased productivity of the discovery process. High-Content technology is now having an impact across life science research from *in vitro* toxicology through oncology and neurobiology and has accelerated the use of new technologies such as RNAi and stem cell models. On **page 26** you will discover innovative tools for neurite morphology and synaptogenesis studies, followed by an application note on an analysis of stem cells differentiation to neurons.

We offer antibodies against key proteins and many other components involved in neurobiology. Go to thermoscientific.com/pierce-antibodies and discover our complete portfolio of immunoreagents, including more than 38,000 antibodies, proteins and peptides, including:

- 2,265 neurobiology reagents
- ~6,000 signal transduction reagents
- ~14,800 cell biology reagents
- ~8,100 reagents for disease and infection

A selection of our primary antibodies for neurobiology appears on **page 31**. Custom antibodies for novel protein targets are also available with antibody-on-demand custom antibody production services. For more information, visit pierce-antibodies.com/custom-antibodies.

Optimized extraction reagent for neuronal tissue and primary cultured neurons

Thermo Scientific N-PER Neuronal Protein Extraction Reagent

N-PER* Neuronal Protein Extraction Reagent is a specialized formulation for the extraction of total protein from neuronal tissue, providing higher yields and better extraction efficiency compared to other reagents while preserving protein function.

With the N-PER Lysis Reagent, protein extraction is completed in less than 30 minutes. For tissue samples, efficient extraction requires mechanical disruption (e.g., Dounce Homogenization, Polytron*) in N-PER Reagent. Typical neuronal protein yields are 70-90µg of protein per mg of brain tissue or 300µg of total protein from 10^6 primary neurons. Neuronal cell lysates prepared with the N-PER Reagent may be used in downstream enzymatic activity assays (e.g., phosphatase, kinase, ATPase assays), immunoassays (e.g., Western blots, ELISAs, RIAs) and protein purification.

Highlights:
- **Optimized** – efficient extraction of total neuronal protein, including membrane proteins, from tissue or primary cultured cells
- **Gentle** – preserves protein function without compromising yield
- **Versatile** – can be supplemented with protease inhibitors, reducing or chelating agents or required cofactors
- **Compatible** – extracts are suitable for use with total protein, enzymatic and immunological assays and protein purification methods

High Efficiency Extraction of Proteins from Neuronal Tissue

N-PER Neuronal Protein Extraction Reagent extracts protein from neuronal tissues more efficiently than other extraction reagents tested, with 1.25- to four-fold higher protein yields (Figure 1). Notably the proprietary formulation of N-PER Reagent increases extraction of integral membrane proteins (N-methyl-D-aspartate receptor type 2B) and membrane associated proteins (Flotillin-1 and Postsynaptic Density Protein 95) compared to other reagents (Figure 2).
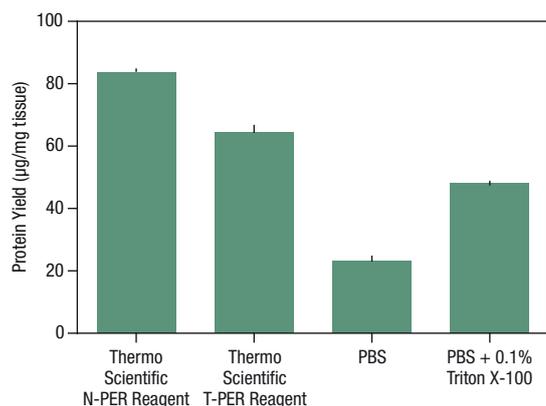

Figure 1. Increased protein yield per mg of neuronal tissue using Thermo Scientific N-PER Reagent. Yields (µg protein/mg tissue) of several extraction reagents are compared to N-PER Reagent using fresh mouse brain tissue and Dounce homogenization. All isolations were performed according to the supplied product instructions. Protein concentrations from cleared supernatants was determined using the Thermo Scientific BCA Protein Assay Kit (Product # 23225).
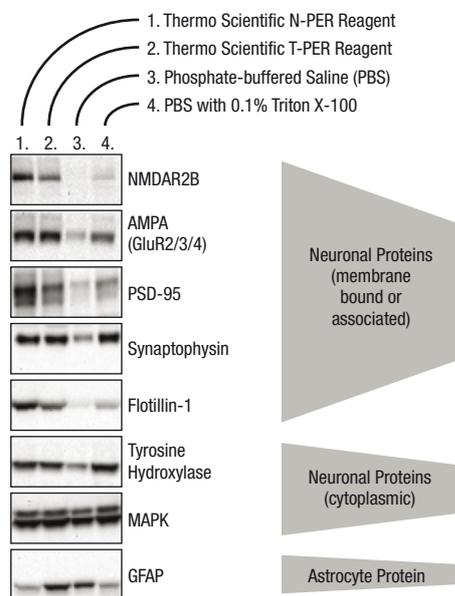

Figure 2. Increased extraction efficiency of specific neuronal proteins using Thermo Scientific N-PER Reagent. Lysates (10µL per well) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antibodies for specific neuronal proteins, including membrane bound/associated proteins. Blots were developed with Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34077). Lane 1: N-PER Neuronal Protein Extraction Reagent, Lane 2: T-PER* Tissue Protein Extraction Reagent, Lane 3: Phosphate-buffered saline, Lane 4: Phosphate-buffered saline with 0.1% Triton* X-100.

Isolation of Active Neuronal Protein using N-PER Reagent

To assess protein function after extraction with N-PER Reagent, we performed several activity assays related to neuronal function. The Rho GTPases (including Rho, Rac, and Cdc42) regulate actin and mictrotubule dynamics, with Rac1 promoting neurite formation and Rho serving as an inhibitor. We evaluated the ability of N-PER Reagent to extract native Rho and Rac GTPases from fresh mouse brain. Lysates were supplemented with 5mM $MgCl_2$ and either 10µM GTPγS or 10µM GDP to keep the native Rho or Rac protein in active and inactive forms, respectively. Active Rho or Rac was isolated from the treated lysates using the Thermo Scientific Active Rho Pull-down and Detection Kit (Product # 16116) or Active Rac Pull-down and Detection Kit (Product # 16118). Both proteins were able to bind GTPγS or GDP in their respective nucleotide binding sites, and the GTP-bound form was capable of interacting with its downstream effector in the pull-down assay (Figure 3), indicating that the function of Rho and Rac1 was maintained.
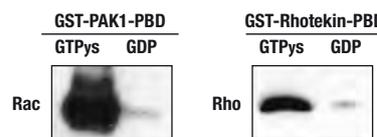

Figure 3. Thermo Scientific N-PER Reagent does not affect activity of GTPases isolated from neuronal tissue. N-PER Reagent was used to isolate brain tissue lysates. Lysates (1mg) were supplemented with 5mM $MgCl_2$ and treated with GTPγS or GDP and were incubated with the indicated GST-PBD and glutathione resin. Active Rho and Rac were isolated following protocols for Active Rho Pull-down and Detection Kit (Product # 16116) and Active Rac Pull-down and Detection Kit (Product # 16118). Half of the eluted sample volumes were analyzed by Western blot and probed using small GTPase-specific antibodies provided in the respective kits.

To determine if protein phosphatases remain active upon extraction, we measured fluorescence of Fluorescein Diphosphate (FDP), a substrate that fluoresces after phosphate cleavage. Phosphorylation of molecules in the brain is important for many neurophysiological events, such as neurotransmitter release, long-term potentiation and neuronal differentiation. In particular, protein tyrosine phosphatases (PTP1B, SHP-2, PTEN and LAR) and serine/threonine phosphatases (PP1 and PP2A) are involved in cell signaling and are classes of enzymes targeted for therapeutic drug development. Although there was not a dramatic difference in phosphatase activity between N-PER Reagent and Thermo Scientific T-PER Tissue Protein Extraction Reagent, both reagents preserved enzymatic activity (Figure 4). Similarly, ATPase/kinase activity is maintained (Figure 5), indicating that researchers could use this reagent for screening small molecules that may affect kinase activity.
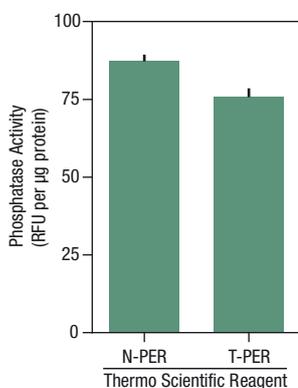
To further evaluate neuronal membrane protein function in N-PER lysates, we measured the protein activity of β-secretase1 (BACE1, β-site APP cleaving enzyme 1), a transmembrane aspartyl protease responsible for the cleavage of amyloid precursor protein (APP), which is associated with beta-amyloid (Aβ) creation. Beta-amyloid peptide is potentially involved in cell signaling, protection from oxidative stress and transcription. Accumulation of Aβ in the brain is also associated with Alzheimer's disease. Beta-secretase and gamma secretase are important in the progression of Alzheimer's disease through the increased generation of Aβ-peptide insoluble aggregates from the miscleavage of amyloid precursor protein. N-PER Reagent solubilizes BACE1 from the membrane, and preserves activity (Figure 6). Brain tissue processed with the N-PER Reagent may be a useful sample source in β-secretase small molecule inhibitor screens.
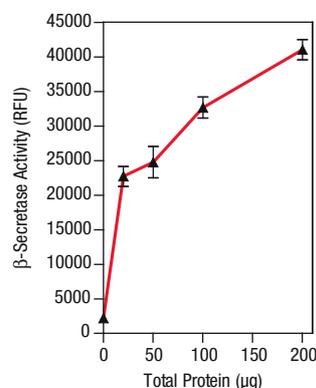

Figure 4. Thermo Scientific N-PER Reagent preserves protein phosphatase activity. Brain tissue lysate (10μg) produced using N-PER Reagent was incubated with a fluorogenic phosphatase substrate for one hour at 37°C. The change in fluorescence (excitation=485nm, emission=520nm) due to substrate hydrolysis was measured using the Thermo Scientific Varioskan Flash Multimode Plate Reader.

Figure 6. Thermo Scientific N-PER Reagent solubilizes active β-Secretase, an integral membrane protease involved in the generation of amyloid-β peptide. Activity of β-secretase was evaluated using a FRET-based assay system (EMD Millipore). Increasing concentrations of brain tissue lysate produced using N-PER Reagent were added to wells containing a fluorogenic peptide substrate, and incubated for one hour at 37°C. Fluorescence was measured at an excitation wavelength of 345nm and an emission of 495nm using a Safire* Plate Reader.
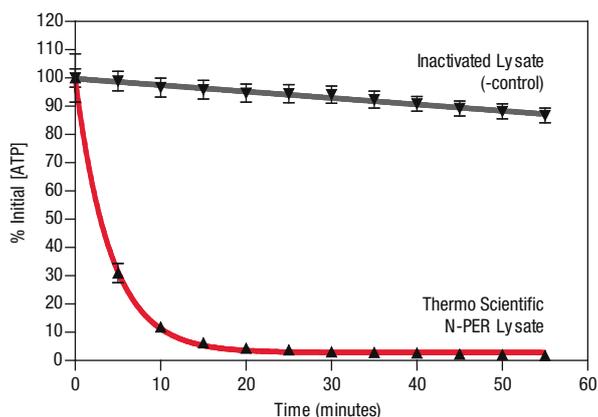

Figure 5. Thermo Scientific N-PER Reagent preserves kinase/ATPase activity. ATPase activity was measured as a function of luciferase chemiluminescence. Brain tissue lysates (10μg) produced using N-PER Reagent were spiked with 5mM ATP. A portion of the lysate was inactivated as a negative control. Lysates were then added to an equal volume of a luciferase-based reaction mix and luminescence was measured with a Varioskan Flash Multimode Plate Reader at five minute intervals over the course of one hour. Values were normalized to initial luminescence values.

Ordering Information

Product #	Description	Pkg. Size
87792	**N-PER Neuronal Protein Extraction Reagent** Sufficient for 10g tissue or up to 200 x 10cm dishes of primary cultured neurons	100mL

neuronal protein isolation

Optimized extraction reagent for neuronal tissue and primary cultured neurons

Thermo Scientific Syn-PER Reagent

The proprietary Syn-PER* Synaptic Protein Isolation Reagent efficiently isolates functional synaptosomes containing active synaptic proteins from neuronal tissue. In addition, Syn-PER Reagent facilitates the study of labile or transient neuronal protein phosphorylation events by stabilizing or preserving these modifications during tissue disruption.

The protocol used with Syn-PER Reagent takes approximately one hour from the start of brain tissue homogenization until collection of the synaptosomal suspension when processing 10 or fewer samples. Syn-PER Synaptic Protein Extraction Reagent efficiently enriches pre-and post-synaptic protein with high yield. The nondenaturing cell lysis reagent is compatible with many downstream applications, including neurotransmitter release assays, enzyme assays (e.g., phosphatase, kinase), immunoassays, various chromatography procedures and electrophoresis. In addition, Syn-PER Reagent preserves phosphoprotein integrity better than most commercially available extraction buffers, even in the absence of phosphatase inhibitors. However, inhibitors such as Thermo Scientific Protease and/or Phosphatase Inhibitor Liquid Cocktails or Tablets can be added just before use to prevent proteolysis or to offer additional protection from the high phosphatase activity normally present in brain tissue.

Highlights:
- **Efficient extraction** – obtain up to 10µg of synaptic protein per milligram of neuronal tissue or 4µg synaptic protein per 35mm dish of primary cultured neurons (10^6 cells)
- **Gentle formulation** – isolate viable synaptosomes, extract native synaptic proteins and preserve phosphoprotein integrity
- **Fast procedure** – obtain synaptosomal suspension in less than one hour
- **Simple protocol** – requires no ultracentrifugation steps

Efficient synaptic protein extraction and enrichment
We compared the total synaptic protein yields of samples prepared with Syn-PER Reagent or a standard homemade buffer obtained from the literature using a general Dounce extraction protocol for fresh mouse brain tissues (Figure 1). The synaptic protein yield in samples obtained using Syn-PER Reagent was about three-fold higher compared to samples prepared with homemade buffer (Figure 2). The total protein concentration of the synaptosome suspension prepared with Syn-PER Reagent was 9.7 ± 1.0µg/mg brain tissue, while with the homemade buffer, we obtained a yield of 3.4 ± 0.8µg/mg brain tissue.
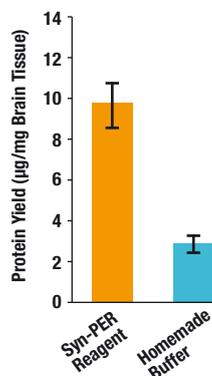
Figure 2. Comparison of protein yield from synaptosome suspension prepared with Thermo Scientific Syn-PER Reagent and homemade buffer. Whole brain or one hemisphere excluding the cerebellums (about 200-400mg) was homogenized as one sample in 10 volumes of Syn-PER Reagent or homemade buffer (protease inhibitors included; Product # 87785) using a 7mL Dounce tissue. The homogenate was centrifuged and supernatant collected. The supernatant was further centrifuged and the pellets, containing synaptosomes, were gently resuspended in their respective buffer. Protein content was estimated using Thermo Scientific BCA Protein Assay Kit (Product # 23225).

To determine the specificity of the synaptosome extraction procedure, we performed Western blot analyses to identify individual synaptic proteins and overall synaptic protein enrichment. The immunoreactivity of N-methyl-D-aspartate receptor 2B subunit (NMDAR2B), PSD95, GluR2/3/4 of α-amino-3hydroxy-5-methyl-4-ioxazolepropionic acid (AMPA) receptor, and synaptophysin in the homogenate, cytosol, and synaptosome suspension was determined (Figure 3). In samples prepared with Syn-PER Reagent, the immunoreactivity of each synaptic protein in the synaptosome suspension is substantially enriched compared to that of the initial homogenate. However, in samples prepared with homemade buffer, little to no enrichment was observed as the signal in the synaptosome suspension is comparable to that of the homogenate. The purity of the synaptosome suspension was further confirmed by probing for the cytosolic proteins, calcineurin and CDK5, and nuclear marker protein HDAC2, which are not significantly represented in the synaptosome fraction, as well as the ubiquitously expressed protein β-actin as a loading control.
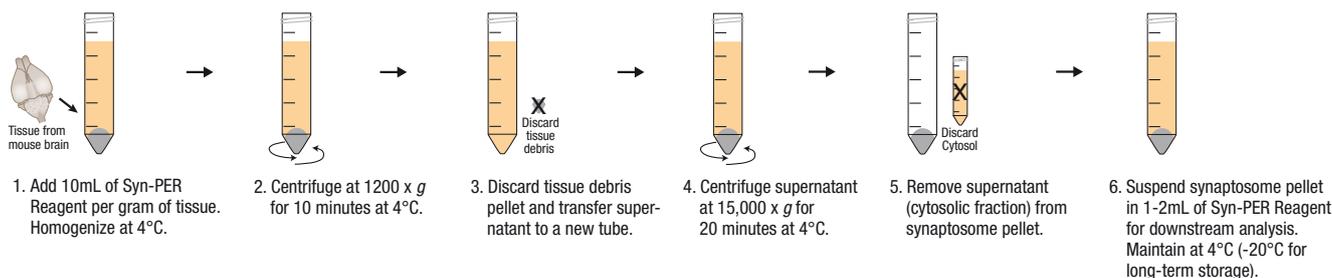

Figure 1. Protocol for the isolation of synaptosomes from mouse brain using Thermo Scientific Syn-PER Reagent or homemade buffer.

1. Add 10mL of Syn-PER Reagent per gram of tissue. Homogenize at 4°C.
2. Centrifuge at 1200 x *g* for 10 minutes at 4°C.
3. Discard tissue debris pellet and transfer supernatant to a new tube.
4. Centrifuge supernatant at 15,000 x *g* for 20 minutes at 4°C.
5. Remove supernatant (cytosolic fraction) from synaptosome pellet.
6. Suspend synaptosome pellet in 1-2mL of Syn-PER Reagent for downstream analysis. Maintain at 4°C (-20°C for long-term storage).

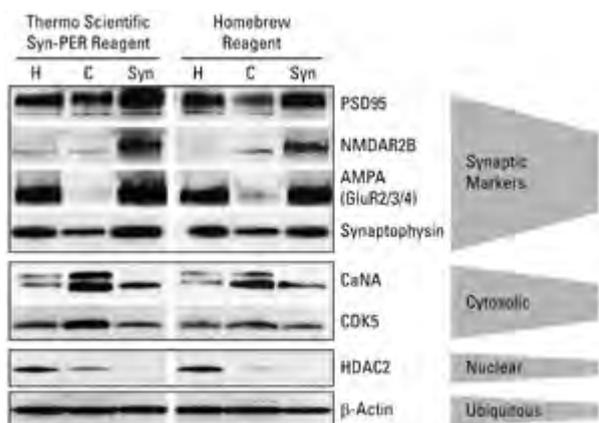*Trademark, see trademark index on the backcover*

Figure 3. Greater enrichment of synaptic proteins is achieved in samples prepared using the Thermo Scientific Syn-PER Reagent than a homebrew reagent. Total protein (10μg) from mouse brain tissue homogenates (H), cytosol (C) fraction and synaptosome suspension (Syn) were analyzed by Western blot. The pre- and post-synaptic protein markers evaluated include synaptophysin, post-synaptic density protein 95 (PSD95), NMDA receptor 2B subunit, and AMPA receptors (GluR2/3/4). Calcineurin, Cdk5 and HDAC2 were purity controls and β-actin served as a loading control. The blots were probed with goat anti-rabbit HRP or goat anti-mouse HRP and detected with Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34077).

Functional synaptosome isolation

To determine if synaptosomes prepared with Syn-PER Reagent are functional, we measured synaptic vesicle endocytosis and exocytosis by monitoring the uptake and release of FM*2-10, a lipophilic styryl fluorescent dye[1]. When the styryl dye FM2-10 was incubated with the synaptosome suspension prepared with Syn-PER Reagent, the endocytotic vesicles internalized FM2-10, resulting in a detectable fluorescent intensity measured at Ex506/Em620 nm (Figure 4). In the presence of calcium, KCl stimulation induced the release of accumulated FM2-10 into solution where FM2-10 is virtually nonfluorescent, and a slow decay of FM2-10 fluorescent intensity over 18 minutes was detected. This result indicates that the Syn-PER Reagent-prepared synaptosome suspension is capable of uptake and release of fluorescent dye FM2-10. Because both endocytosis and exocytosis are highly controlled biological processes regulated by a variety of synaptic proteins, the results of the FM2-10 uptake and release assay also demonstrates that proteins in the Syn-PER Reagent-prepared synaptosome suspension are functional. Therefore, Syn-PER Reagent-isolated synaptosomes provide a useful model with which to study endocytosis and exocytosis in synaptic vesicles, as well as synaptic transmission.
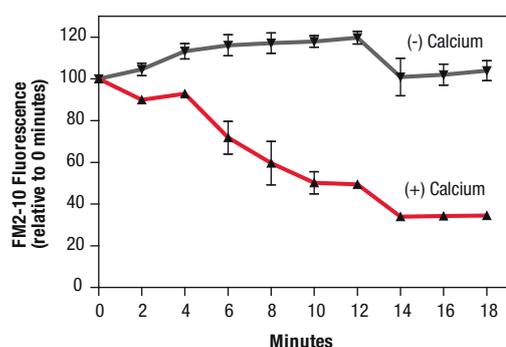

Figure 4. Ca²⁺-dependent and KCl-evoked release of FM2-10 in synaptosomes prepared using Thermo Scientific Syn-PER Reagent. Synaptosomes were resuspended in HBSS either plus or minus 1.2 mM $CaCl_2$. The suspensions were then incubated with 100μM FM2-10 for 15 minutes. The release of FM2-10 was induced by the addition of 30mM KCl. Release of accumulated FM2-10 was then monitored at Ex506/Em620 nm as a decrease in fluorescent intensity upon release of the dye into solution where FM2-10 is no longer fluorescent. Arrow indicates the time-point of adding depolarizing agent 30 mM KCl. Each point is the mean ± SD of two samples.

Phosphoprotein preservation significantly improved

To compare protein phosphorylation levels between samples prepared with Syn-PER Reagent and commercial lysis buffers, antibodies specifically recognizing phospho-ERK (Thr202/Tyr204), phospho-GluR2 (Try869/Tyr873/Try876) of AMPA receptor, and phospho-PSD95 (Tyr236/Tyr240) were used. Western blots detected higher immunoreactivity for each phosphorylated protein in fresh mouse brain homogenates (Figure 5A) and synaptosome suspensions (Figure 5B) prepared with Syn-PER Reagent than in those homogenates prepared with commercial detergent-containing lysis buffers one and two. The levels of total ERK immunoreactivity were comparable among samples prepared with Syn-PER Reagent and both commercial lysis buffers. These data suggest that protein phosphorylation levels are significantly better preserved when brain tissues are homogenized and synaptosome suspensions are prepared using Syn-PER Reagent.
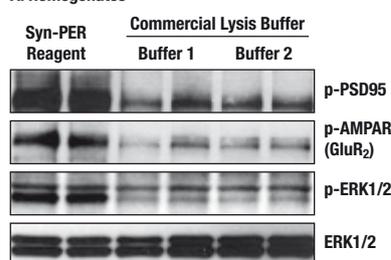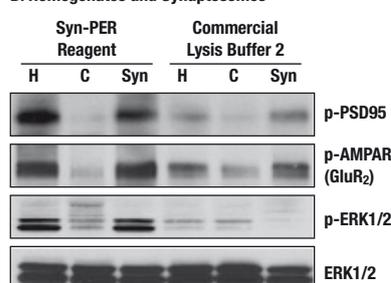

Figure 5. Thermo Scientific Syn-PER Reagent provides better preservation of phosphoprotein immunoreactivity than other commercial lysis buffers. Western blot comparison of immunoreactivity of phosphoproteins, p-PSD95, p-GluR2 of AMPA receptor, and p-ERK1/2, between samples prepared with Syn-PER Reagent and commercial lysis buffers in homogenates (H) (Panel A) and both homogenates, cytosol fraction (C) and synaptosome suspension (Syn) (Panel B). Equal amounts of total protein (10–20μg/lane) were resolved on denaturing 2-10% SDS-polyacrylamide gels. Western blots were performed with the appropriate antibodies and bands were visualized using Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34080).

References:
1. Baldwin, M.L., et al. (2003). Two modes of exocytosis from synaptosomes are differentially regulated by protein phosphatase types 2A and 2B. J Neurochem. 85:1190-9.
2. Bai F and Witzmann. (2007). Synaptosome proteomics. Subcell Biochem. 43:77-98.
3. Salter M.W., et al. (2009). Regulation of NMDA receptors by kinases and phosphatases. Biology of the NMDA Receptor. 7:123-48.

Ordering Information

Product #	Description	Pkg. Size
87793	Syn-PER Synaptic Protein Extraction Reagent Sufficient for 10g tissue or 500 x 35mm dishes of primary cultured neurons	100mL

GTPase pull-down and detection kits

Powerful and simple assay kits for studying signal transduction

Thermo Scientific Active GTPase Pull-down and Detection Kits

Active GTPase Pull-down and Detection Kits profile the activation of small GTPases by isolating them via their specific downstream effectors.

Active (GTP-bound) small GTPases are difficult to directly detect because no primary antibodies are available that are specific for the active forms. To circumvent this problem, the kits precede detection with pull-down purification to differentiate active GTPases from their inactive, GDP-bound conformation based on their affinity for downstream effector proteins.

This affinity provides a method to distinguish the transient up- or down-regulation of the active populations of GTPases from global changes in GTPase expression. These transient changes in activation could be the experimental result of exposing cells to growth factors or other small-molecule stimulators or inhibitors, or due to changes that occur during cell growth, differentiation, tumorigenesis and metastasis.

Active GTPases interact with specific downstream effector proteins. These specific protein-protein interactions can be exploited to capture active GTPases by using the effector proteins as the bait in pull-down assays. The GTPase protein-binding domains (PBDs) of these effector proteins are expressed as functional GST-fusion proteins that can be immobilized to glutathione agarose, facilitating the capture and enrichment of the active forms of their cognate GTPases. Although effector proteins often interact with one or more active GTPase, each GTPase can be individually identified and the relative quantity determined by Western blot analysis after the pull-down assay.

The GTPase pull-down assay procedure is streamlined with the Active GTPase Pull-Down and Detection Kits, which are available for Arf1, Arf6, Cdc42, Rac1, Rap1, Ras and Rho small GTPases. The pull-down affinity purification step is easily performed in convenient spin columns. After washing, the bound GTPase is recovered by eluting the GST-fusion protein from the glutathione resin. The purified GTPase is detected by Western blot using a specific antibody supplied in each kit. Relative differences in active GTPase signal can be quantified by densitometry or CCD camera. The GTPase pull-down procedure is already optimized for Western blot analysis using Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate and HRP-conjugated secondary antibodies (available separately).

Assay summary

Performing pull-down assays with the Active GTPase Pull-Down and Detection Kits is straightforward. Firstly, whole cell lysates are prepared from cultured cells using the lysis buffer provided in the kit. Lysate (containing active and inactive GTPase) is incubated with the GST-protein binding domain (PBD) fusion protein from the respective downstream effector protein and glutathione resin. This specific interaction enables isolation of the target active (GTP-bound) GTPase. Unbound lysate proteins, including inactive or GDP-bound GTPase, are removed using the spin columns. The active GTPase population is recovered from the glutathione resin using SDS-PAGE loading buffer and analyzed by Western blot.

Highlights:

- **Highly sensitive and accurate** – reagents, antibodies and Western blot procedure ensure accurate controls and semi-quantitative results
- **Convenient** – no need to express and purify your own GST-PBD fusion proteins or use expensive antibody-based affinity resins
- **Easy to use** – achieve immediate success in a two-hour assay
- **Efficient** – spin columns separate liquid from resin to prevent sample loss and cross-contamination
- **Complete** – kits include reagents for affinity purification, controls, cell lysis buffer and antibodies for Western blot detection
- **Validated** – kits are functionally tested to ensure quality and performance
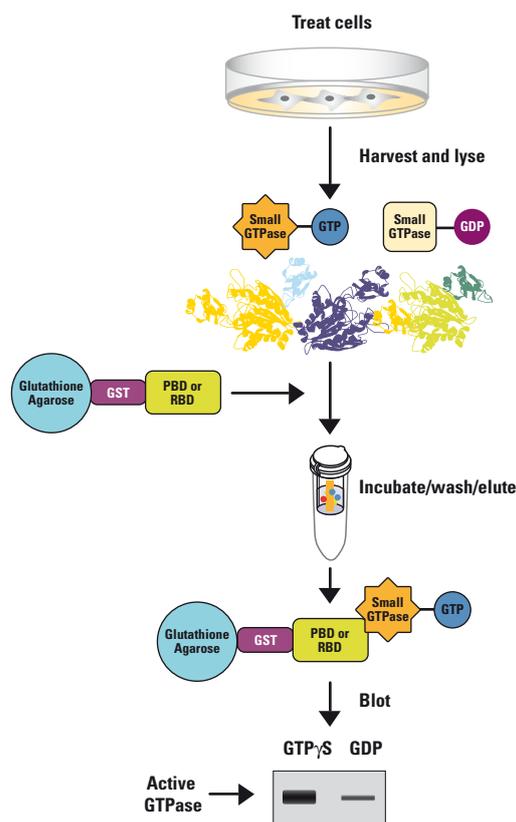- **Compatible** – kits work with a variety of cell types (e.g., mouse, rat and human)

Figure 1. Thermo Scientific Active GTPase Pull-Down and Detection Kit assay summary.

Ordering Information

Product #	Description	Pkg. Size
16116	Active Rho Pull-Down and Detection Kit	30-rxn kit
16117	Active Ras Pull-Down and Detection Kit	30-rxn kit
16118	Active Rac1 Pull-Down and Detection Kit	30-rxn kit
16119	Active Cdc42 Pull-Down and Detection Kit	30-rxn kit
16120	Active Rap1 Pull-Down and Detection Kit	30-rxn kit
16121	Active Arf1 Pull-Down and Detection Kit	30-rxn kit
16122	Active Arf6 Pull-Down and Detection Kit	30-rxn kit

Pull-down kits enrich active GTPases from cell or tissue lysates

Suzanne M. Smith, M.S.; Kay K. Opperman, Ph.D.; Rizwan Farooqui, Ph.D.; and Barbara J. Kaboord, Ph.D.; Thermo Fisher Scientific

The Ras superfamily of small GTPases serve as molecular switches to control diverse eukaryotic cellular behaviors, including cell growth, differentiation and motility. Consequently, small GTPases are involved in several disease states such as cancer and metabolic disorders.[1,2] GTPases are active when bound to guanosine triphosphate (GTP) and inactive when the triphosphate is hydrolyzed to guanosine diphosphate (GDP). The Active GTPase Pull-down and Detection Kits enable GTPase activation studies by preferentially enriching their active form. These kits contain a GST-protein binding-domain (PBD or RBD) fusion that is selective for active Rho, Ras, Rac1, Cdc42, Rap1, Arf1 or Arf6 (Table 1).

GTPase	Downstream effector binding domain	Cellular function
Rho	GST-Rhotekin-RBD	Filopodia, lamellipodia formation, and stress fibers[3]
Ras	GST-Raf1-RBD	Cell proliferation/differentiation[4]
Rac1	GST-Pak1-PBD	Filopodia, lamellipodia formation, and stress fibers[3]
Cdc42	GST-Pak1-PBD	
Rap1	GST-RalGDS-RBD	Cell proliferation/differentiation[5]
Arf1	GST-GGA3-PBD	Assembly of coat proteins onto budding vesicles on trans-golgi network and endosomes[6,7]
Arf6	GST-GGA3-PBD	Membrane traffic, actin remodeling and structural organization at the cell surface[6,7]

Table 1. Each active GTPase kit includes a GST fusion of the protein-binding domain.

This pull-down method is based on the affinity of known downstream effector proteins for the active forms of specific GTPases. The respective protein-binding domain (PBD) of these downstream effectors is expressed as a GST-fusion protein (Table 1). When immobilized on glutathione agarose resin, the PBD will bind active, GTP-bound GTPase from a cell lysate. The pulled-down active GTPase is detected via Western blotting. As a control, cell lysates can be treated with GTPγS, which is a non-hydrolyzable analog of GTP. This method traps all GTPases in the active form and results in high GTPase enrichment. As a negative control, cell lysates are treated with an excess of GDP to shift the majority of GTPase to the inactive state.
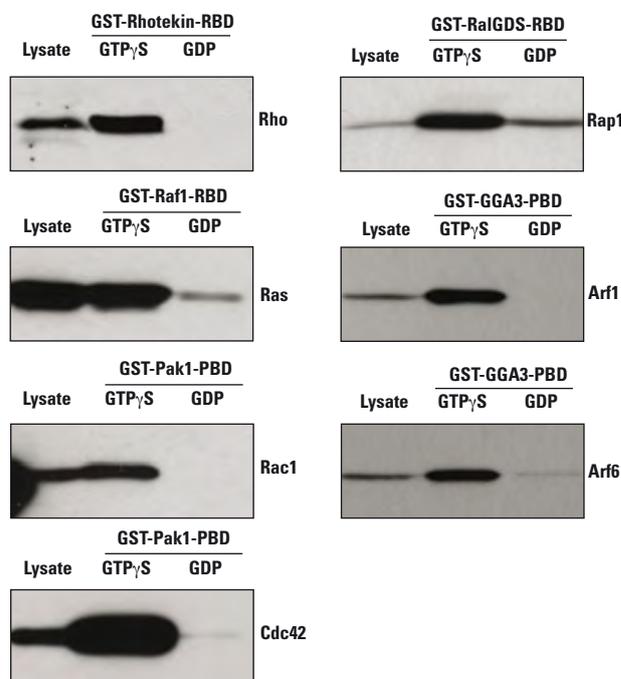

Figure 2. Specific detection of active Rho, Ras, Rac1, Cdc42, Rap1, Arf1 and Arf6 by Western blotting. NIH 3T3 cell lysate treated with GTPγS or GDP was incubated with the appropriate GST binding domain and immobilized glutathione resin. Eluted samples and a portion of the lysate were analyzed by Western blot using GTPase-specific antibodies.

To determine the specificity and function of the GTPase pull-down and detection kits, NIH 3T3 cell lysate was incubated with either GTPγS or GDP to activate or inactivate endogenous GTPases, respectively. The specific GST-PBD or -RBD was used to pull down active Rho, Ras, Rac1, Cdc42, Rap1, Arf1 or Arf6. A strong signal is detected in the GTPγS-treated lysate; however, minimal or no signal is detected in the GDP-treated lysate (Figure 2). These results illustrate the specificity of the PBD for active GTPases.

The pull-down of endogenous active small GTPases after growth factor or serum stimulations was highly effective in a variety of cell types derived from different species (Figure 3). Changes in the GTPase activities can be detected in time-course studies and differ with cell type and treatment. Because total GTPase levels in each lysate are constant, the amount of GTPase pulled down in each assay reflects activation rather than changes in GTPase expression levels. The activity profiles detected are similar to those reported in the literature.[8-11] These results demonstrate the effectiveness of the GTPase pull-down and detection kits for monitoring sensitive changes in activity using time-dependent activity assays.

GTPase pull-down and detection kits

A — HeLa — 0 15 30 60 120 240 min EGF — Rho-GTP / Total Rho — Rho Activity

B — NIH 3T3 — 0 5 15 30 60 240 min PDGF — Ras-GTP / Total Ras — Ras Activity

C — NS1 — 0 1hr 1 2 3 4 days NGF — Rac1-GTP / Total Rac1 — Rac1 Activity

D — MDCK — 0 1 2 4 6 hr HGF — Arf1-GTP / Total Arf1 — Arf1 Activity

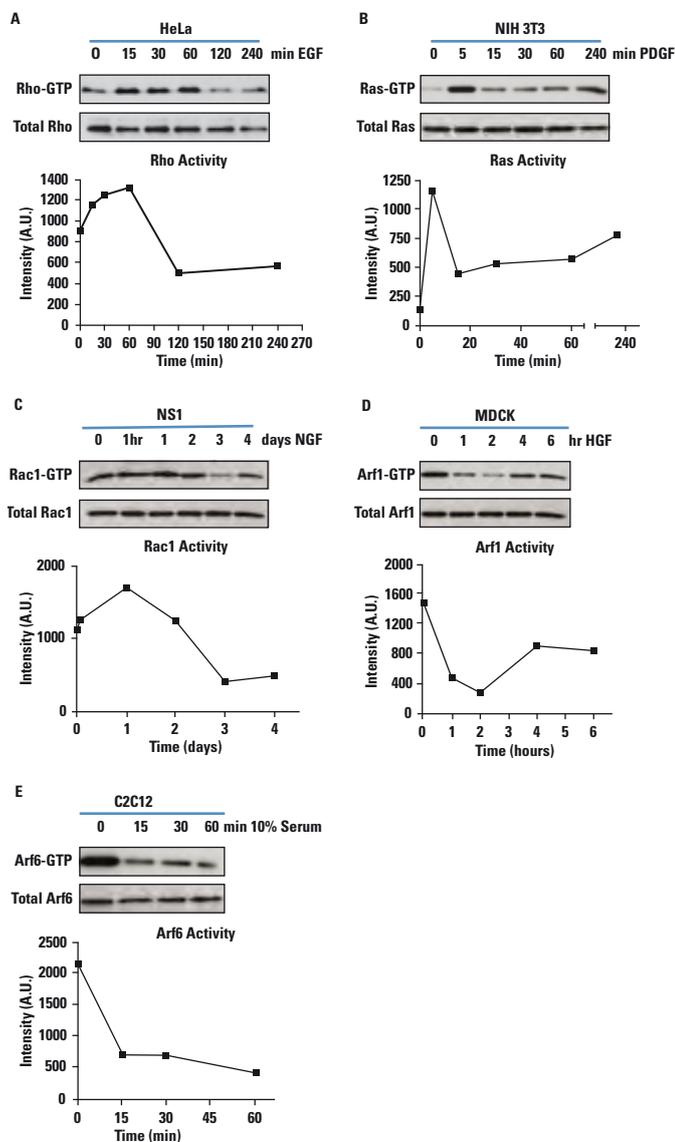**E** — C2C12 — 0 15 30 60 min 10% Serum — Arf6-GTP / Total Arf6 — Arf6 Activity

Figure 3. Specific, induced changes in the level of endogenously activated GTPases from a variety of cell types are easily monitored by the pull-down assay. In each panel, the top Western blot shows the level of active GTPase isolated by pull-down assay; the lower Western blot shows the total amount of expressed GTPase in the lysate. Densitometry was performed on the Western blots and plotted graphically for each system. **Panel A**: Rho activity in HeLa (human) cells stimulated with EGF. **Panel B**: Ras activity in NIH 3T3 (murine) cells stimulated with PDGF. **Panel C**: Rac1 activity in NS1 (rodent) cells stimulated with NGF. **Panel D**: Arf1 activity in MDCK (canine) cells stimulated with HGF. **Panel E**: Arf6 activity in C2C12 (murine) cells stimulated with serum.

Methods

Cell culture and treatments

HeLa cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) to ~70% confluency and then starved in 1% FBS medium for 24 hours before stimulation with 100ng/mL of epidermal growth factor (EGF) for the indicated times. NIH 3T3 cells were grown in DMEM supplemented with 10% FBS to ~70% confluency and starved in 0.1% FBS medium for 24 hours. Platelet-derived growth factor (PDGF) was added at 50ng/mL for the indicated times. NS1 cells were grown in RPMI supplemented with 10% FBS to ~70% confluency and nerve growth factor (NGF, 50ng/mL) was added for the indicated times. MDCK cells were grown in EMEM supplemented with 10% FBS to ~70% confluency and starved in serum-free medium for 48 hours before stimulation with 50ng/mL of hepatocyte growth factor (HGF) at indicated times. C2C12 cells were grown in DMEM supplemented with 10% FBS to ~70% confluency and were starved in serum-free medium for 48 hours before adding 10% serum at the indicated times.

Active GTPase pull-down and detection

NIH 3T3 cells were lysed on the culture plate with 1mL lysis/binding/wash buffer. The clarified cell lysate (500µg) was treated with either GTPγS (positive control) or GDP (negative control). The treated lysates (or 1mg of the endogenous time-course lysates) were incubated with 400µg GST-Rhotekin-RBD (for active Rho), 80µg GST-Raf1-RBD (for active Ras), 20µg GST-Pak1-PBD (for active Rac1 or Cdc42), 20µg GST-RalGDS-RBD (for active Rap1) or 100µg GST-GGA3-PBD (for active Arf1 or Arf6). Half of each elution was analyzed by SDS-PAGE and detected by Western blot using the specific GTPase primary antibody.

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Measure activation of small GTPases via their specific downstream effectors
Pull-down kits enrich active GTPases from cell or tissue lysates

Kay K. Opperman, Ph.D.; Suzanne M. Smith, M.S.; Hai Yan Wu, Ph.D.; Barbara J. Kaboord, Ph.D.; and Rizwan Farooqui, Ph.D.; Thermo Fisher Scientific

The differentiation of neuronal cells requires signaling pathways that are responsive to the extracellular matrix as well as extensive remodeling of the cytoskeleton. GTPases are critical for these processes. The Ras GTPase family acts as membrane-associated signal transducers; the Rho GTPase family regulates actin and microtubule dynamics. The cellular location of the GTPases and their respective effector binding proteins contribute to cellular differentiation. In this study, Rho and Ras GTPases were assayed for activity and cellular localization using the components of the Thermo Scientific Active GTPase Pull-down and Detection Kits. In addition to using the kits for pull-down assays, the GTPase antibody and GST-tagged effector binding domain were used for immunofluorescent localization. Stimulation of NS-1 cells (neuronal cell line derivative of PC-12) with neuronal growth factor (NGF) resulted in a time- and location-dependent activation of the GTPase targets that were tested. These results could be correlated to staining patterns in primary rat cortical neurons. The kit enabled detection of active GTPase activity in neuronal cells and was an effective tool for studying cellular localization of active GTPase activity.

Introduction

Differentiated cells have highly specified roles guided by cascades of protein interactions. In these cascades, small GTPases help link cell surface receptors to the actin cytoskeleton, guide interaction with other cells and the extracellular matrix, and direct the delivery and internalization of lipids and proteins. In neurogenesis, cytoskeleton rearrangement and microtubule organization are critical for the initial disruption of cell shape and bud formation for neurite outgrowth and extension. To study neuronal outgrowth *in vitro*, undifferentiated neuronal cell lines are stimulated with neuronal growth factor (NGF) and monitored for a time period. NGF signaling occurs through the tyrosine kinase receptor (TrkA) and activates Ras GTPase at the membrane. Additional Ras and Rho GTPases are activated after signaling of Ras via PI3 kinase, resulting in active Rap1, RalA and the Rho GTPases. The Ras, Rap1 and RalA GTPases serve as upstream signal transducers; however, the Rho GTPases (Rac1, Cdc42, and RhoA) act antagonistically and affect the actin skeleton and microtubules, transcriptional activation, and membrane trafficking. Rac1 and Cdc42 promote neurite formation and RhoA inhibits neural differentiation. The intricate regulation of GTPases determines the neuronal cell differentiation fate (Figure 1).[1-3]
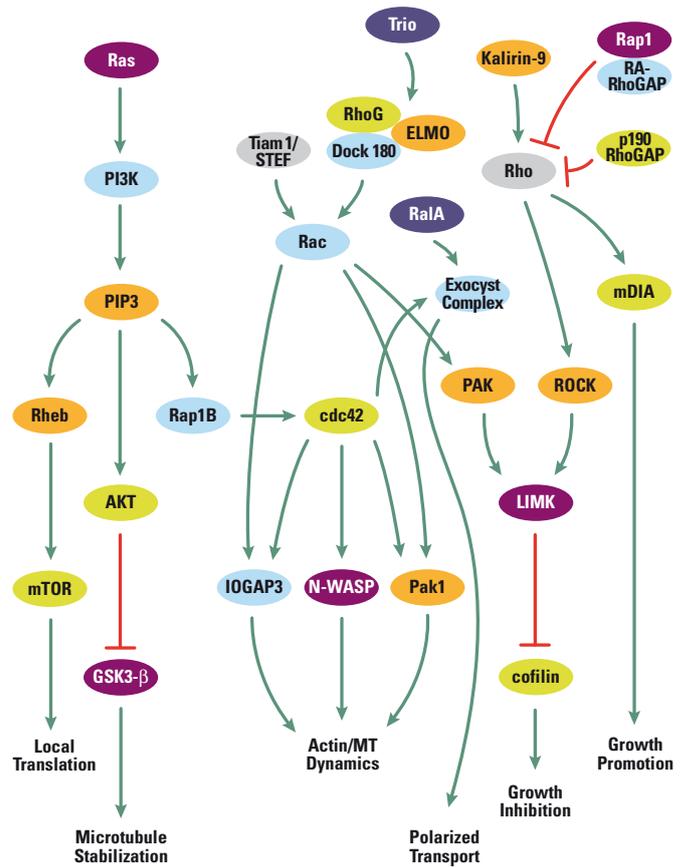

Figure 1. Role of GTPases in neurogenesis. A complex balance of interacting signaling pathways controls neuronal cell differentiation. Green arrows: promotes changes in cell morphology; Red lines: abrogates changes in cell morphology.

GTPase pull-down and detection kits

Regulation of neural differentiation is dependent on both the signaling cascade and the spatial location of the GTPases in context to their respective effector binding proteins. After NGF stimulation, Rac1 is recruited to the membrane to form membrane ruffles and then localizes to the distal half of the neurites during differentiation. Cdc42 is present in the microspikes projecting from the tips. Both Rac1 and Cdc42 trigger neuronal differentiation through Pak1 kinase. If RhoA is activated, RhoA forms a thick ring-like structure at the cell periphery to prevent recruitment of Rac1 to the cell membrane, resulting in neurite retraction.[3] The negative regulation of neurite extension by RhoA is dependent on Rho kinase (ROCK). After the initial signaling event that triggers differentiation, each GTPase may induce both positive and negative regulation of neurite growth and axonal signaling (Figure 2) to provide cellular fluidity for signaling and regulatory roles in polarization, extension, guidance and regeneration.
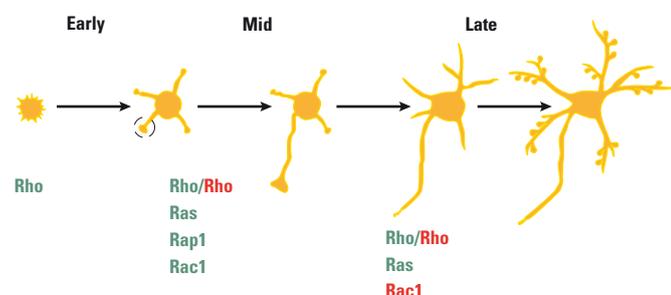

Figure 2. Stages of neuronal development. The GTPases that were assayed in this study are listed below the stages. Green: positive; Red: negative.

We stimulated neuronal NS-1 cells with NGF and studied Rho and Ras family GTPase activity using the Active GTPase Pull-down and Detection Kits. Active GTPase activity was assessed by a functional pull-down assay using a GST fusion of the downstream effector protein that binds only the active form of the GTPase. The spatial distribution of active GTPases was determined by immunofluorescent staining using the GST-PBD protein and anti-GTPase antibody supplied in the kit.

Results and Discussion

Based on the functional pull-down results, GTPases are differentially regulated with time. Ras activity peaks at day one and two, and Rac1 and Rho activity is present at early time points and diminishes with time. Activity of RalA showed no significant changes (Figure 3). Immunofluorescent staining revealed that Rac1 is present at the membrane ruffles and extends throughout the neurite extension. Colocalization with Pak1 at the periphery and in the neurite tips suggests "active" Rac1 in these regions (Figure 4). Similar staining patterns of Rac1 and Pak1 PBD were obtained with primary differentiated rat cortical neurons (Figure 5). These results loosely correlate with what is reported in earlier studies.[1-3] Rho, however, is localized as a thick ring around the cell body and in the perinuclear region. After stimulation, colocalization with rhotekin is in the perinuclear region and does not extend into the neurite extensions, which is consistent with previous results. Ras is also present in the perinuclear region and at the cell periphery, consistent with its function in cell signaling from the membrane to the nucleus. Colocalization with its effector binding domain Raf1 suggests that active Ras is present in the nodes of the neurite extensions as well as in the cell body (Figure 4).
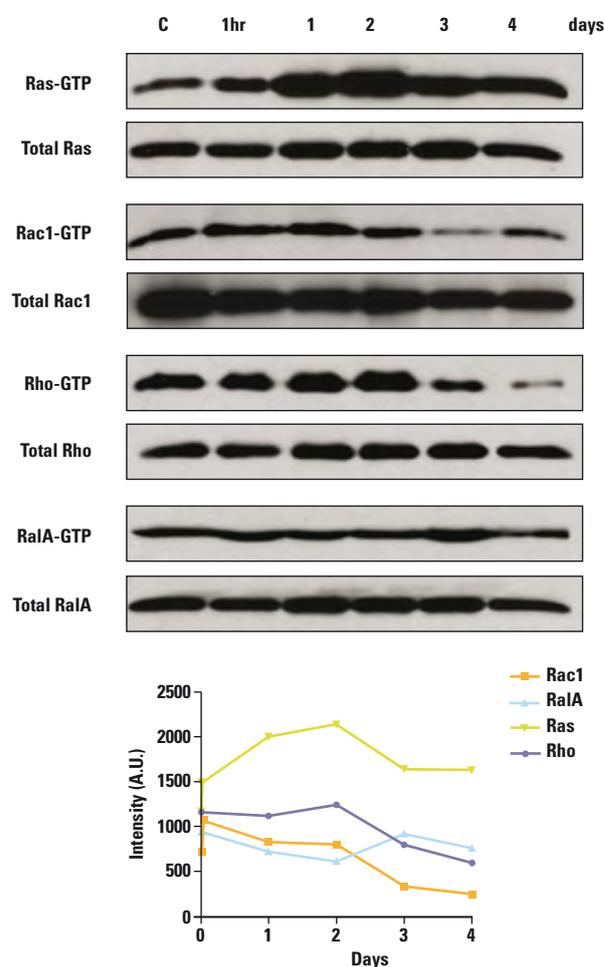

Figure 3. Assay of active GTPase activity by functional pull-down. Active GTPases were detected in NGF-stimulated NS-1 cells as described in Methods. Spot densitometry was performed on each scanned blot and normalized to scale. The graph summarizes the induction of Ras, Rac1, Rho, and RalA for a four-day period.

Conclusion

Using the Active GTPase Pull-down and Detection Kits enabled visualization of GTPase activity during the course of differentiation and GTPase cellular localization. The GST-tagged GTPase effector binding domain should only stain "active GTPases"; however, some of the effectors bind multiple proteins and GTPases. Therefore, co-localization of the GTPase antibody with the respective binding domain suggests the cellular location of "active" GTPases. Co-localization studies provide a better understanding of spatial activity during differentiation; however, more optimization of the effector binding domains is necessary for better specificity and lower background.
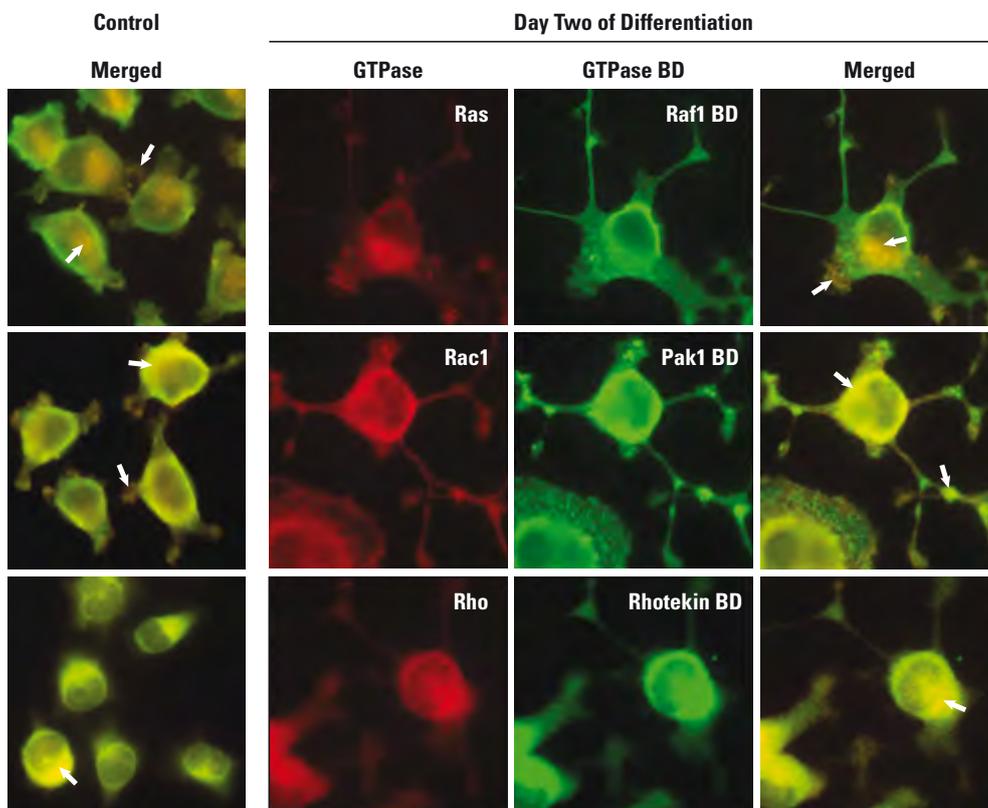
Control	Day Two of Differentiation		
Merged	GTPase	GTPase BD	Merged

Ras | Raf1 BD

Rac1 | Pak1 BD

Rho | Rhotekin BD

Figure 4. Use of GTPase binding domains as antibody alternatives localizes GTPases activity in differentiated neuronal cells. NS-1 cells were grown and treated with NGF as described in Methods. Images of GTPase and GTPase-binding domain (BD) staining of day two differentiated cells are monochrome. Merged images of non-treated (control) and day two differentiated cells are multicolored. GTPases were detected using DyLight* 549-conjugated secondary antibodies. The GTPase effector binding proteins were detected using DyLight 488-conjugated anti-GST antibody. Arrows denote areas of colocalization.
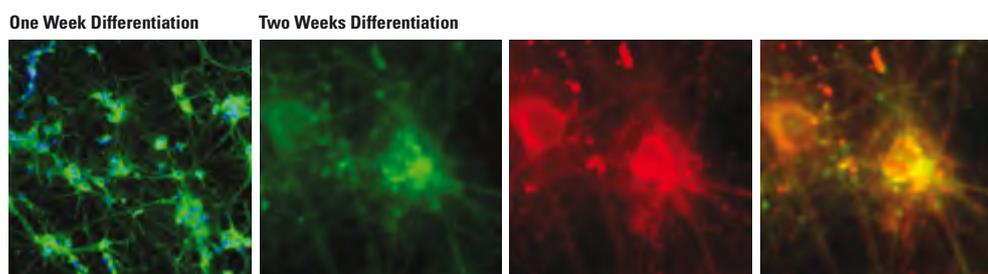
One Week Differentiation | Two Weeks Differentiation

Figure 5. Rac1 and Pak1 exhibit similar localization patterns in rat primary cortical neurons. Fresh cortex tissue from E18 Sprague-Dawley rat (BrainBits, Springfield, IL) was used to culture primary neurons in poly-D-lysine-coated 12-well slides. Cells were co-stained with Rac1 and Pak1 as described in Methods.

Methods

Cell culture

NS-1 cells were cultured in RPMI media containing 15% FBS, pen/strep, and HEPES buffer on collagen IV-coated plates or on cell culture-treated eight-well chamber slides (BD Biosciences). At ~80% confluency, cells were stimulated with 50ng/mL NGF (EMD Biosciences) or untreated. For functional pull-down assays, cells were harvested at one hour and one, two, three and four days post-treatment using the lysis buffer supplied in the kit. Active GTPases were detected from fresh cell lysates (1mg total protein) by Western blot as per kit instructions. For immunofluorescent staining, media was gently removed and replaced with warmed 4% paraformaldehyde for 20-30 minutes at 37°C to preserve neuronal structure. Slides were stored at 4°C until stained.

Immunofluorescent staining

After fixation, cells were permeabilized with 0.05% Triton X-100 in phosphate-buffered saline (PBS) for 15 minutes, and blocked for 30 minutes in 5% fetal bovine serum (FBS) in PBS. Cells were incubated with the GST-effector binding domain fusion protein (Raf1, rhotekin, or Pak1) at 50-200µg/mL for one hour,

rinsed and stained with anti-GTPase antibody (Ras, pan-Rho and Rac1, respectively) for one hour at room temperature (1:250-1:500 dilution). Cells were washed and stained with Thermo Scientific DyLight 549 Dye conjugated to goat anti-mouse or goat anti-rabbit IgG (1:500 dilution), Thermo Scientific DyLight 488 Dye conjugated to anti-GST antibody (1:500 dilution) and Hoechst 33342 (DNA stain) for 30 minutes at room temperature. Cells were washed and dehydrated using a 70, 80, 90, 100 ethanol series. Coverslips were mounted using VECTASHIELD* Mounting Media, and images were acquired using the Axio Observer (Carl-Zeiss, Inc.) inverted microscope (63X objective) and AxioVision* Software Module.

References:
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kinase enrichment kits and probes

Reagents for the selective capture and enrichment of kinases using active-site probes

Thermo Scientific Pierce Kinase Enrichment Kits

Pierce* Kinase Enrichment Kits utilize ActivX ATP or ADP Probes to covalently label the active site of ATPases, including chaperones and metabolic enzymes, to enable their selective enrichment using a desthiobiotin tag.

ActivX ATP and ADP Probes feature an amine-reactive nucleotide analog and a desthiobiotin (biotin analog) tag that facilitates selective labeling of lysines in the kinase active site and then subsequent enrichment and recovery of labeled protein. These features allow identification and profiling of target enzyme classes across samples or assessment of the specificity and affinity of enzyme inhibitors.

Highlights:
- **Specific** – label only the conserved active-site lysines of nucleotide-binding proteins
- **Flexible** – use for *in vitro* labeling of ATPase enzymes derived from cells or tissues
- **Compatible** – use with Western blot or mass spectrometry (MS) workflows (Figure 2)

Applications:
- Profile small-molecule binding affinities and active-site inhibition in a dose-dependent manner
- Identify dozens to hundreds of inhibitor targets and off-targets from tissues, cells and subcellular proteomes
- Enrichment of enzymes based on function

Product Details:
- Thermo Scientific ActivX Desthiobiotin-ATP and -ADP are nucleotide derivatives that covalently modify the active site of enzymes at conserved lysine residues in the nucleotide binding site. The structure of these probes consists of a modified biotin (desthiobiotin) attached to the nucleotide through a labile acyl-phosphate bond. Desthiobiotin is a biotin analog that binds less tightly to biotin-binding proteins resulting in binding that is easily reversed by biotin displacement, low pH or heat denaturation (Figure 1).
- Depending on the position of the lysine within the enzyme active site, either desthiobiotin-ATP or -ADP might be better for labeling specific ATPases. Both desthiobiotin-ATP and -ADP probes can be used to selectively enrich, identify and profile target enzyme classes or assess the specificity of enzyme inhibitors. Because many ATPases and other nucleotide-binding proteins bind nucleotides or inhibitors even when they are enzymatically inactive, the desthiobiotin probes allow profiling of both inactive and active enzymes in a complex sample (Figure 3). Preincubation of samples with small-molecule inhibitors that compete for active sites can be used to determine inhibitor binding affinity. Active-site nucleotide probes also can be used to identify inhibitor off-targets.
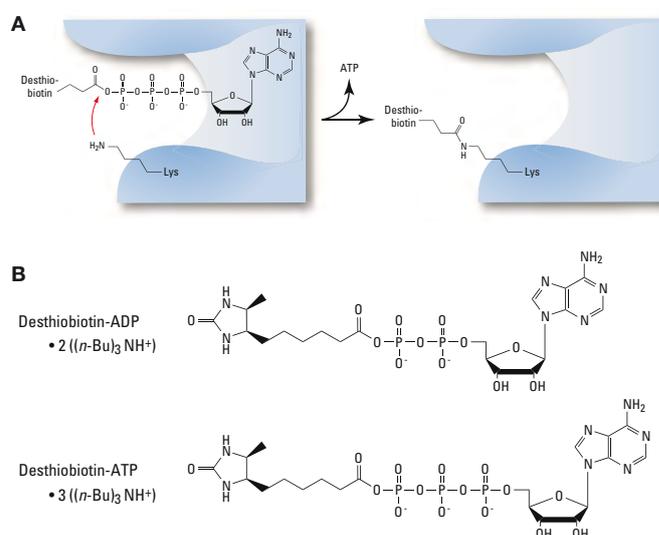
Figure 1. Mechanism and chemical structures of Thermo Scientific Active Site Probes for kinases and other ATPases. Panel A: Nucleotide analogues bind to the active sites of ATPases and the biotin affinity tag is irreversibly transferred to highly conserved lysine residues in the active site. **Panel B**: Structures of desthiobiotin nucleotide analogues. Desthiobiotin binding to streptavidin is easily reversible under acidic elution conditions, allowing high recovery of labeled proteins and peptides. Desthiobiotin is attached to the nucleotide through a labile acyl phosphate linkage, allowing efficient desthiobiotin label transfer to amines near the active site. ATP and ADP nucleotide analogues label a complementary set of ATPases, which is likely due to differences in the proximity of the acyl phosphate linkage to conserved lysines near the active site.
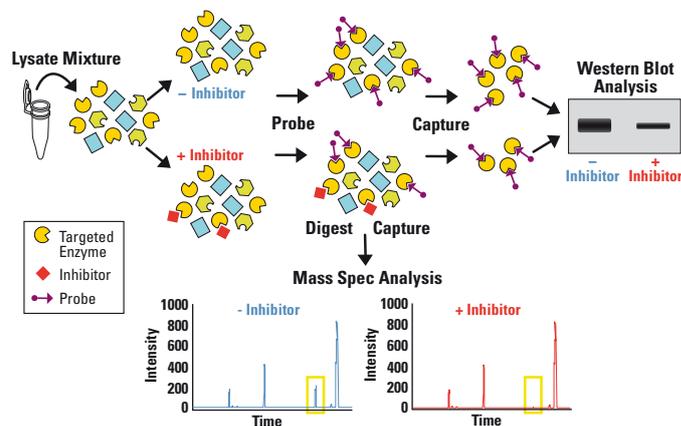
Figure 2. Assessment of active-site labeling can be accomplished by Western blot or mass spectrometry (MS). For the Western blot workflow, desthiobiotin-labeled proteins are enriched, analyzed by SDS-PAGE and detected with specific antibodies. For the MS workflow, desthiobiotin-labeled proteins are reduced, alkylated and enzymatically digested. Only the desthiobiotin-labeled, active site peptides are enriched for LC-MS/MS analysis. Both workflows can be used to determine inhibitor target binding, but the MS workflow also can identify global inhibitor targets and off-targets and provide higher throughput for quantitative assays.
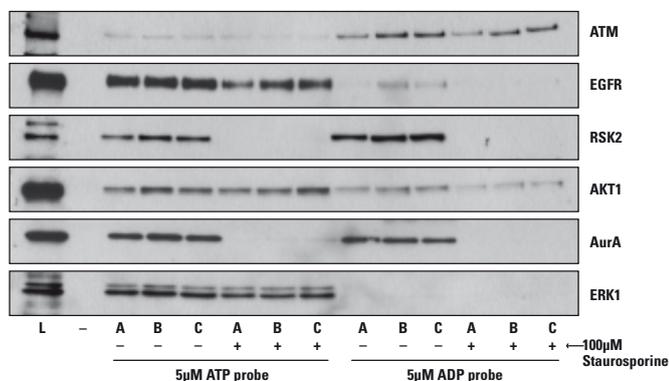
Figure 3. Comparison of desthiobiotin ATP and ADP probe labeling of kinases using the Western blot workflow. A549 cell lysates (500µg) were treated with (+) or without (-) 100µM staurosporine before labeling with 5µM of each probe in triplicate (A-C). Desthiobiotin-labeled proteins were denatured and enriched using streptavidin agarose before separation by SDS-PAGE and Western blotting with specific kinase antibodies. Unlabeled lysate (-/-) was used as a control to show streptavidin pulldown specificity.

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Ordering Information

Product #	Description	Pkg. Size
88310	**Pierce Kinase Enrichment Kit with ATP Probe** Formulation: Multi-component kit Sufficient For: 16 pull-down assays	16-rxn Kit
88311	**ActivX Desthiobiotin-ATP Probe** Formulation: Desthiobiotin-ATP, MW 1259	16 x 12.6µg
88312	**Pierce Kinase Enrichment Kit with ADP Probe** Formulation: Multi-component kit Sufficient For: 16 pull-down assays	16-rxn Kit
88313	**ActivX Desthiobiotin-ADP Probe** Formulation: Desthiobiotin-ADP, MW 994	16 x 9.9µg

Now available: GTPase and Fluorophosphonate Enrichment Kits and Probes

The Thermo Scientific Pierce GTPase Enrichment Kit with ActivX GTP Probe enables selective labeling and enrichment of small GTPases and large G-protein subunits. The Thermo Scientific ActivX Desthiobiotin-GTP Probe structure consists of a modified biotin attached to the nucleotide by a labile acyl-phosphate bond.

Fluorophosphonate (FP) probes are another class of active site probes that are specific for active serine hydrolases. Because these reagents label only active enzymes, they are able to monitor enzymatic activity in addition to being used for studying inhibitor binding affinities.

For more information, go to www.thermoscientific.com/pierce and search for ActivX products.

kinase enrichment kits and probes

Kinase inhibitor profiling of TrkA- and TrkB- expressing neuroblastoma cell lines using desthiobiotin nucleotide probes

Ryan D. Bomgarden, Chris L. Etienne, Rosa Viner, Michael M. Rosenblatt, Eugene Cichon and John C. Rogers
Thermo Fisher Scientific

Neuroblastoma is a cancerous tumor of nerve tissue that develops in children and is the most common tumor found in children younger than one year of age. In the U.S. alone, approximately 500 children are diagnosed with neuroblastoma each year. Clinical behavior of this disease is variable; some cases show spontaneous regression, whereas others are untreatable despite maximally tolerable chemotherapy. Although much genetic data has been collected on these tumors, the biology responsible for this disease is not well understood. Current evidence suggests that the tropomyosin-receptor kinase (Trk) family of neurotrophin receptors plays a critical role in neuroblastoma phenotypes and prognosis. Tumors expressing TrkA are usually benign and prone to spontaneous regression, whereas tumors expressing TrkB have poor prognosis and are associated with MYCN amplification.

Lestaurtinib is an orally active small-molecule inhibitor that has been used to treat acute myeloid leukemia (AML)[2] and is currently being evaluated for the treatment of other cancers, including neuroblastoma. Lestaurtinib is a staurosporine derivative that has shown specificity for receptor tyrosine kinases including the tropomyosin receptor kinases TrkA, TrkB, and TrkC and FMS-like tyrosine kinase 3 (FLT3).[3] However, lestaurtinib specificity for other kinases has not been fully evaluated.

Here, we examined the relative expression and inhibition of kinases in SH-SY5Y neuroblastoma cells stably expressing TrkA or TrkB. We employed a proteomic approach using desthiobiotin nucleotide probes to specifically capture and profile the kinome of each cell line using mass spectrometry to identify labeled kinase active-site peptides (Figure 1 - page 16).[4] In addition, we assessed staurosporine and lestaurtinib inhibition of protein kinases using kinase active-site probes in combination with Tandem Mass Tag (TMT) reagents and validated our results using a parallel Western blot workflow (Figure 2 - page 16). Finally, because this approach allows for the specific enrichment of adenine nucleotide binding proteins of interest, an SRM-based workflow could be used to enable targeted quantification as an alternative means of validating the relative expression and inhibition of the kinases, and may be employed as a follow-up to this study.

Goal

To identify staurosporine and lestaurtinib kinase targets and measure kinase expression and inhibition in TrkA and TrkB expressing neuroblastoma cells using kinase active site probes in combination with TMT reagent quantitation.

Experimental Conditions

Sample Preparation

Cell culture and kinase labeling: TrkA and TrkB stable SH-SY5Y cell lines were grown in RPMI media with 10% FBS and 0.3mg/mL G418. Cells (107) were lysed using Thermo Scientific Pierce IP Lysis Buffer and desalted using Thermo Scientific 7K Zeba Spin Desalting Columns according to the manufacturer's protocol.[5] Cell lysates (1mg) were labeled with 5μM of desthiobiotin-ATP or -ADP for 10 minutes at room temperature. For inhibitor selectivity analysis, cell lysates were pretreated with 100μM of staurosporine (S) or lestaurtinib (L) before labeling with desthiobiotin nucleotide probes. Labeled proteins were denatured with 6M urea and captured with Thermo Scientific High Capacity Streptavidin Agarose Resin for two hours. Bound proteins were washed and eluted by boiling in sample buffer before SDS-PAGE separation and Western blotting using specific antibodies.

Active-site peptide capture and TMT reagent labeling: Desthiobiotin-ATP labeled proteins were reduced and alkylated before buffer exchange into digestion buffer (20 mM Tris pH 8.0, 2 M urea). Each sample was tryptically digested for two hours; active-site peptides were captured with streptavidin agarose resin and eluted using 50% acetonitrile/0.1% TFA.[6] For Tandem Mass Tag (TMT) reagent labeling, active-site-labeled peptides were first lyophilized to dryness and then labeled with Thermo Scientific TMTsixplex reagents (TrkA cells with 126-128 and TrkB cells with 129-131 reagent) for one hour at room temperature, quenched with hydroxylamine4, desalted using Thermo Scientific PepClean C18 Spin Columns, and combined in one-to-one ratios before LC-MS/MS analysis.

LC/MS Analysis using Thermo Scientific LTQ Orbitrap XL Mass Spectrometer (Tune 2.5.5 SP1)

LC separation

Column:	Magic C18 column (5 µm, 20 cm x 75 µm ID, Michrom)
Mobile phases:	0.1% formic acid in water (eluent A); 0.1% formic acid in acetonitrile (eluent B)
Gradient:	5% – 10% B in 10 minutes, 10% – 35% B in 90 minutes
Flow:	300 nL/minute

Mass Spectrometry

Mass spectrometer:	LTQ* Orbitrap XL with nanospray source
MS resolution:	60,000
MS2 resolution:	7,500
FT MS AGC target:	5e5
FT MS/MS AGC target:	1e5
IT MS/MS AGC target:	1e4
Injection time:	FT MS/MS: 300 ms; IT MS/MS: 100 ms
Full MS mass range *(m/z)*:	400 – 1,600
MS/MS mass range *(m/z)*:	100 – 2,000
Acquisition method:	Full MS with Orbitrap detection followed by top three Data Dependent IT CID and top three Data Dependent HCD events
Collision energy:	HCD: 40%; CID: 35%
Isolation width:	2 amu
Dynamic exclusion:	Repeat count: 1 Exclusion list size: 500 Exclusion duration: 90 s Early expiration: disabled Exclusion mass width: low/high 10 ppm
Charge state screening:	On; +1 and unassigned charge states rejected
Monoisotopic precursor:	Enabled

Data Analysis

The raw data files were searched using Thermo Scientific Proteome Discoverer software version 1.2 with Mascot* v. 2.3 (Matrix Sciences Ltd., London, UK) and SEQUEST* search engines against SwissProt database (v 57.15). Figure 4 on page 20, shows the TMT quantitation data analysis workflow used in the Proteome Discoverer* software.

For the protein identification search the following settings were selected:

Taxonomy:	*Homo sapiens*
Maximum number of missed cleavages:	4
Precursor mass tolerance:	10 ppm
Fragment mass tolerance:	0.8 Da (CID), 20 mmu (HCD)
Instrument type:	ESI-FTICR
Modifications static:	Carbamidomethyl cysteine (C) TMT 6 (N-term)
Modifications dynamic:	Desthiobiotin lysine (K) TMT 6 lysine (K) Oxidation methionine (M)
Scoring peptide cut off score:	10 MUDPIT scoring Protein relevance threshold: 20 Protein relevance factor: 1
DTA generation conditions:	Total intensity threshold: 100 Minimum peak count: 8 S/N threshold: 1.5 Precursor mass range: 800 – 8,000 Da

The identified proteins were filtered using medium and high confidence on the peptide level, and peptide rank one (5% FDR). To quantify TMT ratios, 10 ppm mass tolerance of reporter ions and the "Apply Quan Value Corrections" feature in Reporter Ions Quantifier tool were used. All results were manually verified and ratios from co-isolating precursors were excluded.

Results and Discussion

Thermo Scientific ActivX Desthiobiotin-ATP and -ADP are nucleotide derivatives that covalently modify the active site of enzymes at conserved lysine residues in the nucleotide binding site (Figure 1B - page 16). The structure of these probes consists of a modified biotin (desthiobiotin) attached to the nucleotide through a labile acyl-phosphate bond. Desthiobiotin is a biotin analog that binds less tightly to biotin-binding proteins resulting in binding that is easily reversed by biotin displacement, low pH, or heat denaturation.

Depending on the position of the lysine within the enzyme active site, either desthiobiotin-ATP or -ADP might be better for labeling specific ATPases. Both desthiobiotin-ATP and -ADP probes can be used to selectively enrich, identify, and profile target enzyme classes or assess the specificity of enzyme inhibitors. Because many ATPases and other nucleotide-binding proteins bind nucleotides or inhibitors even when they are enzymatically inactive, the desthiobiotin probes allow profiling of both inactive and active enzymes in a complex sample. Preincubation of samples with small-molecule inhibitors that compete for active sites can be used to identify inhibitor targets and determine inhibitor binding affinity (Figure 2 - page 16).
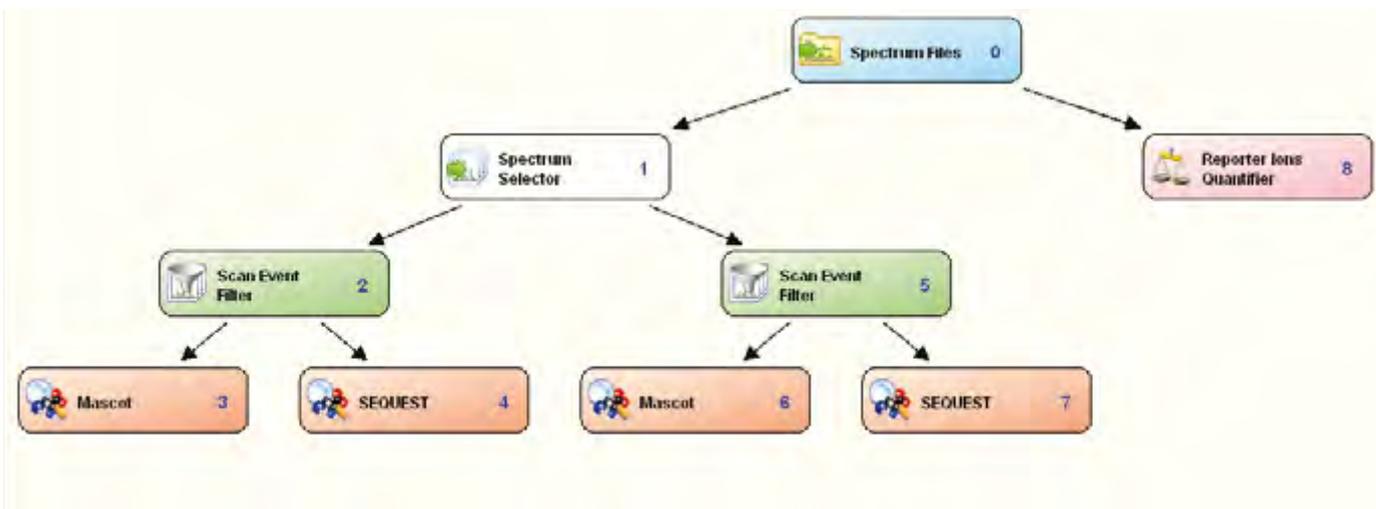
Figure 4. Workflow in Thermo Scientific Proteome Discoverer software for CID/HCD relative quantitation of isobarically labeled peptides/proteins.

In our study, we used these probes in combination with TMT reagent quantitation to measure kinase expression and inhibition in TrkA and TrkB expressing neuroblastoma cells. TMT reagents are isobaric MS quantitation reagents that produce a unique reporter ion during MS/MS analysis of peptides.[6] Figure 5 shows the TMT sixplex reagent workflow for peptide labeling after sample treatment and digestion. This approach enabled the identification and quantification of more than 150 protein kinases of which more than 20 kinases showed greater than 50% inhibition by both staurosporine and lestaurtinib (Table 1). These data are comparable to previously published results when a different kinase enrichment approach such as Kinobeads* (Cellzome AG) was employed.[7] In addition, 10 kinases were found to have greater than two-fold increased expression in TrkA versus TrkB cells. Although TrkA and TrkB expression was confirmed by Western blot, we did not detect TrkA or TrkB kinase active-site peptides using mass spectrometry. Since both of these tyrosine kinases are membrane proteins, subcellular fractionation may be necessary for identification and quantitation.

Figure 6 shows the difference in kinase expression as determined by Western blot of TrkA vs TrkB cell lysates after kinase enrichment using a desthiobiotin-ATP probe. Both PLK1 and AurA showed higher expression in TrkB cells compared to TrkA cells. Cdk5 had over two-fold lower expression in TrkB expressing cells and correlated well with TMT Reagent quantitation by mass spectrometry (Figure 7). Treatment of cell lysates with staurosporine or lestaurtinib before labelin with desthiobiotin-ATP probe showed significant reduction in RSK2, PLK1, AurA, and Cdk5 kinase enrichment for both inhibitors. In contrast, we observed no differences in expression or inhibition for mitogen-activated protein kinase 3 (ERK 1/2) and the chaperone hsp90. Again, these data correlated well with changes in relative peptide abundance after each drug treatment (Figure 7). In addition, we were able to identify proteins that showed specific inhibition in response to each drug such as PRPK (TP53 kinase) for lestaurtinib and DCLK3 for staurosporine (Figure 7 and Table 1). Similar experiments can be performed on the LTQ Orbitrap Velos mass spectrometer using settings from the referenced publication by Zhang, *et al.*[8]
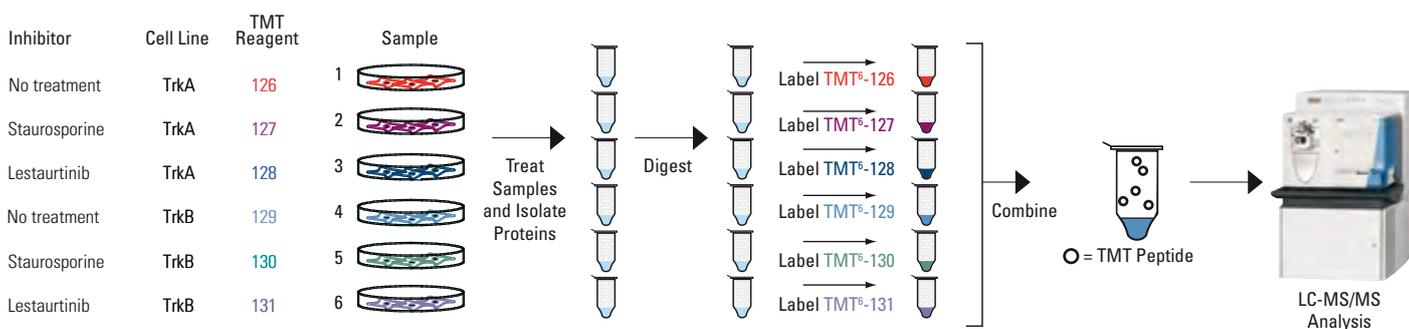
Inhibitor	Cell Line	TMT Reagent	Sample					
No treatment	TrkA	126	1			Label TMT⁶-126		
Staurosporine	TrkA	127	2			Label TMT⁶-127		
Lestaurtinib	TrkA	128	3	Treat Samples and Isolate Proteins	Digest	Label TMT⁶-128		
No treatment	TrkB	129	4			Label TMT⁶-129	Combine	
Staurosporine	TrkB	130	5			Label TMT⁶-130		
Lestaurtinib	TrkB	131	6			Label TMT⁶-131		LC-MS/MS Analysis

Figure 5. Thermo Scientific TMT Reagent workflow and labeling scheme.

ID	Kinase	TrkA/TrkB	Staurosporine, 100 µM		Lestaurtinib, 100 µM	
			TrkA	TrkB	TrkA	TrkB
Q9UQ88	CDK11a	3.07	NI	NI	NI	NI
O14757	CHK1	2.05	30%	40%	100%	100%
P17252	PKCa	2.16	NI	NI	30%	30%
Q96GX5	MASTL	2.10	80%	80%	70%	70%
Q13131	AMPKa1, AMPKa2	2.19	100%	100%	100%	100%
Q6PHR2	ULK3	2.54	100%	100%	100%	100%
Q00535	CDK5	2.44	>90%	100%	100%	100%
P41240	CSK	2.16	>90%	>90%	>80%	>80%
Q13188	MST2	2.06	100%	100%	100%	100%
Q9C098	DCLK3	2.00	100%	100%	NI	NI
O75116	ROCK2	0.41	NI	NI	NI	NI
Q96S44	PRPK	1.35	NI	NI	70%	70%
P16591	FER	1.5	100%	100%	100%	100%
O95835	LATS1	1.3	100%	100%	100%	100%
Q9Y2U5	MAP3K2	1.12	>60%	>60%	>60%	>60%
Q99759	MAP3K3	1.12	>60%	>60%	>60%	>60%
Q02750	MAP2K1, MAP2K2	1.02	>90%	>90%	>80%	>90%
P23443	RPS6KB1	1.18	70%	70%	NI	NI
Q9HC98	NEK6	1.13	NI	NI	NI	NI
O43683	BUB1	1.5	>90%	>90%	>90%	>90%
O94804	STK10	1.5	>60%	>75%	>70%	>70%
Q13555	CAMK2G	1.20	>90%	>90%	>90%	>90%
Q13557	CAMK2D	1.01	>75%	>75%	>75%	>75%
P36507	MAP2K2	1.4	100%	100%	100%	100%
Q15078	CD5R1	1.69	>80%	>80%	>80%	>80%

PLK1
hsp90
RSK2
AurA
ERK1/2
CDK5

- S L   - S L
TrkA   TrkB

Figure 6. Kinase expression and inhibitor assessment by Western blot.

Turn page for Figure 7.

Table 1. Kinase abundance and inhibition measured using Thermo Scientific TMT Reagent workflow.
"NI" = no inhibition, "100%" = 100% inhibition.

Conclusions

- Combining active-site probe labeling with TMT Reagents resulted in the identification and relative quantitation of over 150 kinases, more than 20 of which showed > 50% inhibition by staurosporine and/or lestaurtinib.
- Lestaurtinib was found to have very broad kinase specificity similar to staurosporine.
- At least 10 kinases were found to have > two-fold increased expression in TrkA versus TrkB cells indicating differences in stable cell line remodeling.
- Mass spectrometry of active-site-labeled peptides correlated with Western blot analysis of labeled proteins.
- An SRM-based workflow could be used as an alternative means of validating the relative expression and inhibition of the kinases.
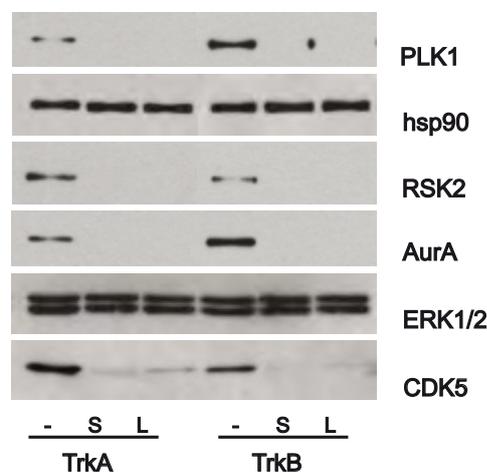
References:
1. Brodeur, G.M., *et al.* (2009). Trk receptor expression and inhibition in neuroblastomas. *Clin Cancer Res* **15(10):**3244-50.
2. Knapper, S., *et al.* (2006) A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood* **108(10):**3262-70.
3. Revill, P., *et al.* (2007). Lestaurtinib. *Drugs of the Future* **32(3):**215.
4. Patricelli, M.P., *et al.* (2007). Functional interrogation of the kinome using nucleotide acyl phosphates. Biochemistry **46:**350-8.
5. Instruction booklet for Pierce Kinase Enrichment Kit, doc.#2345.
6. Instruction booklet for TMT Mass Tagging kits and reagents, doc.#2073.
7. Savitski, M.M., *et al.* (2010). Targeted data acquisition for improved reproducibility and robustness of proteomic mass spectrometry assays. *JASMS* **20:**1425-34.
8. Zhang, T. *et al.* (2010) Robust and sensitive relative quantitation of TMT labeled E. coli digest by dual cell linear ion trap-orbitrap Hybrid Mass Spectrometer. *58th ASMS conference on Mass Spectrometry.*
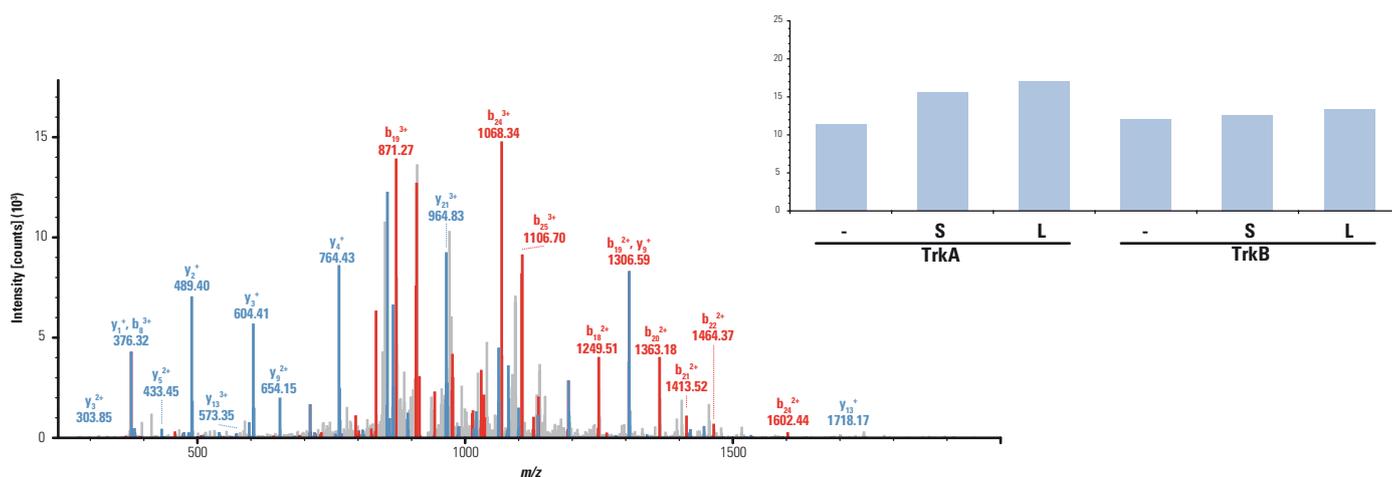
PRPK **FLSGLELVkQGAEAR (ATP site)**
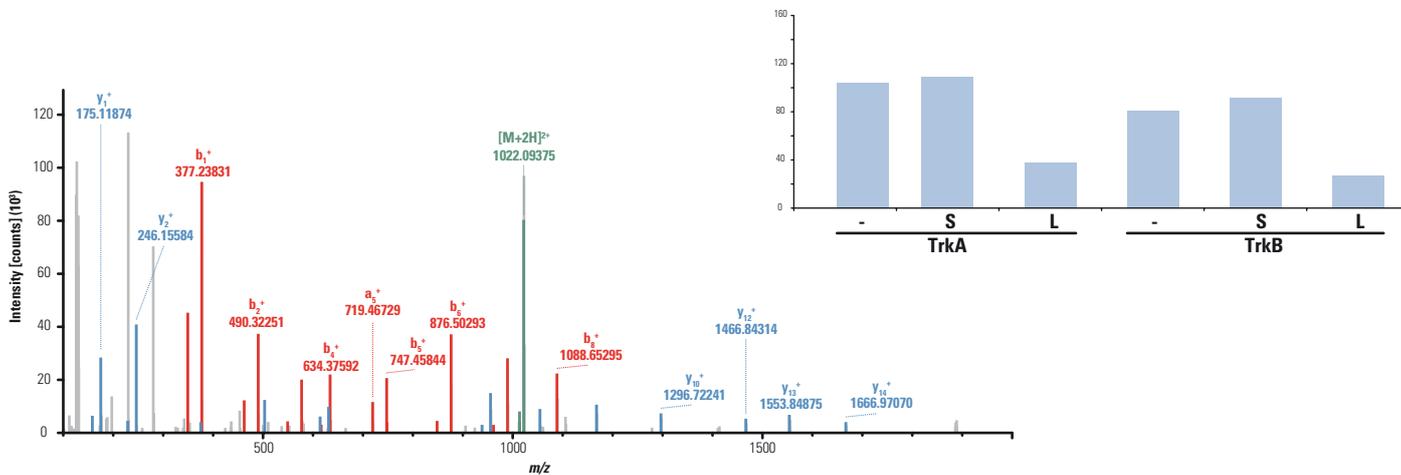
ERK1/2 **YIHSANVLHRDLkPSNLLINTTCDLK (K2 site)**

CDK5 **DLkPQNLLINR (K2 site)**
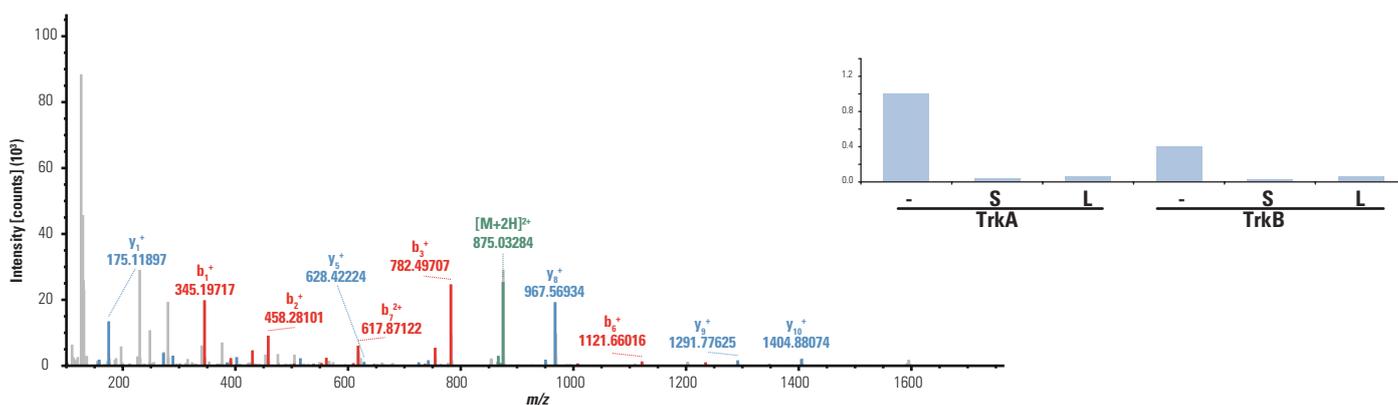

Figure 7. Kinase expression and inhibitor assessment by mass spectrometry of kinase active-site peptides.

Thermo Scientific TMT Isobaric Mass Tagging Kits and Reagents

Amine-reactive TMT Isobaric Mass Tagging Kits and Reagents enable quantitative tandem labeling of proteins extracted from cells and tissues for identification and analysis by mass spectrometry.

Each isobaric Tandem Mass Tag Reagent is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter. The reagents label peptides prepared from cell-based or tissue samples, either two samples for the duplex kit or six samples for the sixplex kit. For each sample, a unique reporter mass results in the MS/MS spectrum, providing a simple means of identification.

Changes in protein expression and post-translational modifications are essential mechanisms of biological regulation and disease. Advancements in mass spectrometry (MS) instrumentation, bioinformatics and quantification methods, such as label-free quantification, metabolic labeling and chemical tagging, now enable researchers to identify and quantitatively analyze thousands of proteins in a given sample with a high degree of accuracy. Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry. Tandem labeling extends the analytic power of isobaric labeling for MS analysis.

Highlights:

- **Powerful** – parallel mass labeling enables protein identification and quantitation from multiple samples of cells, tissues and biological fluids
- **Robust** – consistent chemistry allows efficient transition from method development to multiplex quantitation, enabling biomarker discovery research
- **Efficient** – amine-reactive NHS-ester activated reagents ensure efficient labeling of membrane and post-translationally modified proteins
- **Flexible** – expandable system allows concurrent multiplexing of up to six different samples in a single experiment (Figure 2)
- **Compatible** – optimized fragmentation and fully supported quantitation with Protein Discoverer* 1.0 for all Thermo Scientific LC MS/MS platforms, such as LTQ XL* and LTQ Orbitrap* XL Systems (Figures 3-4)

Applications:

- Protein identification and quantitation from multiple samples of cells, tissue or biological fluids
- Protein expression profiling of normal vs. disease states or control vs. treated
- Multiplex up to six different samples concurrently in a single experiment
- Quantitative analysis of proteins for which no antibodies are available
- Identification and quantitation of membrane and post-translationally modified proteins
- Identification and quantification of hundreds to thousands of proteins in a single experiment
- The TMT Isobaric Mass Tagging Kits and Reagents enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry

Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry. They are small chemical molecules with identical structure that covalently attach to the free amino termini of lysine residues of peptides and proteins, thereby labeling various peptides in a given sample (Figure 1).

Our Tandem Mass Tag (TMT) Reagents are uniquely designed to enable a rapid and cost-effective transition from method development to high-throughput protein quantitation. The tags consist of TMTzero*, the TMTduplex* set and the TMTsixplex* set (Figure 3). The TMT0 tag allows testing and optimization of sample preparation, labeling, fractionation and MS fragmentation for peptide identification and reporter detection without using the more costly isotope-labeled compounds. The TMTduplex reagent set allows two-plex protein profiling for small studies. The TMTsixplex reagent set allows six-plex protein profiling for multiple conditions, including time courses, dose responses, replicates or multiple sample comparisons. Each TMT tag is based on the same chemical structure, eliminating the need to modify labeling conditions or HPLC separation conditions between experiments.

During the MS/MS analysis, each isobaric tag produces a unique reporter ion signature that makes quantitation possible. In the first MS analysis, the labeled peptides are indistinguishable from each other; however, in the tandem MS mode during which peptides are isolated and fragmented, each tag generates a unique reporter ion. Protein quantitation is then accomplished by comparing the intensities of the six reporter ions in the MS/MS spectra.

The tags are provided as standalone sets or in optimized kit formats containing all necessary reagents and controls for maximum flexibility, convenience and reliability. The TMT Reagents combined with Thermo Scientific instruments and software provide a complete and integrated solution to perform absolute quantitation of target proteins (Figure 2, 4).
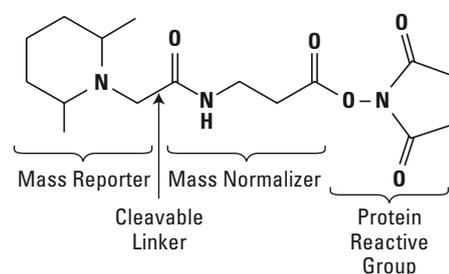

Figure 1. Structural design of Thermo Scientific Tandem Mass Tag. Mass reporter: Each member has a unique mass and reports sample-specific abundance of a labeled peptide during MS/MS analysis. **Cleavable linker**: Preferentially fragments under typical MS/MS conditions to release the mass reporter. **Mass normalizer**: Each member has a unique mass that balances the mass reporter, ensuring the same overall mass for all tags in a set. **Reactive group**: Reactive NHS ester provides high-efficiency amine-specific labeling of proteins/peptides.

protein identification and quantitation

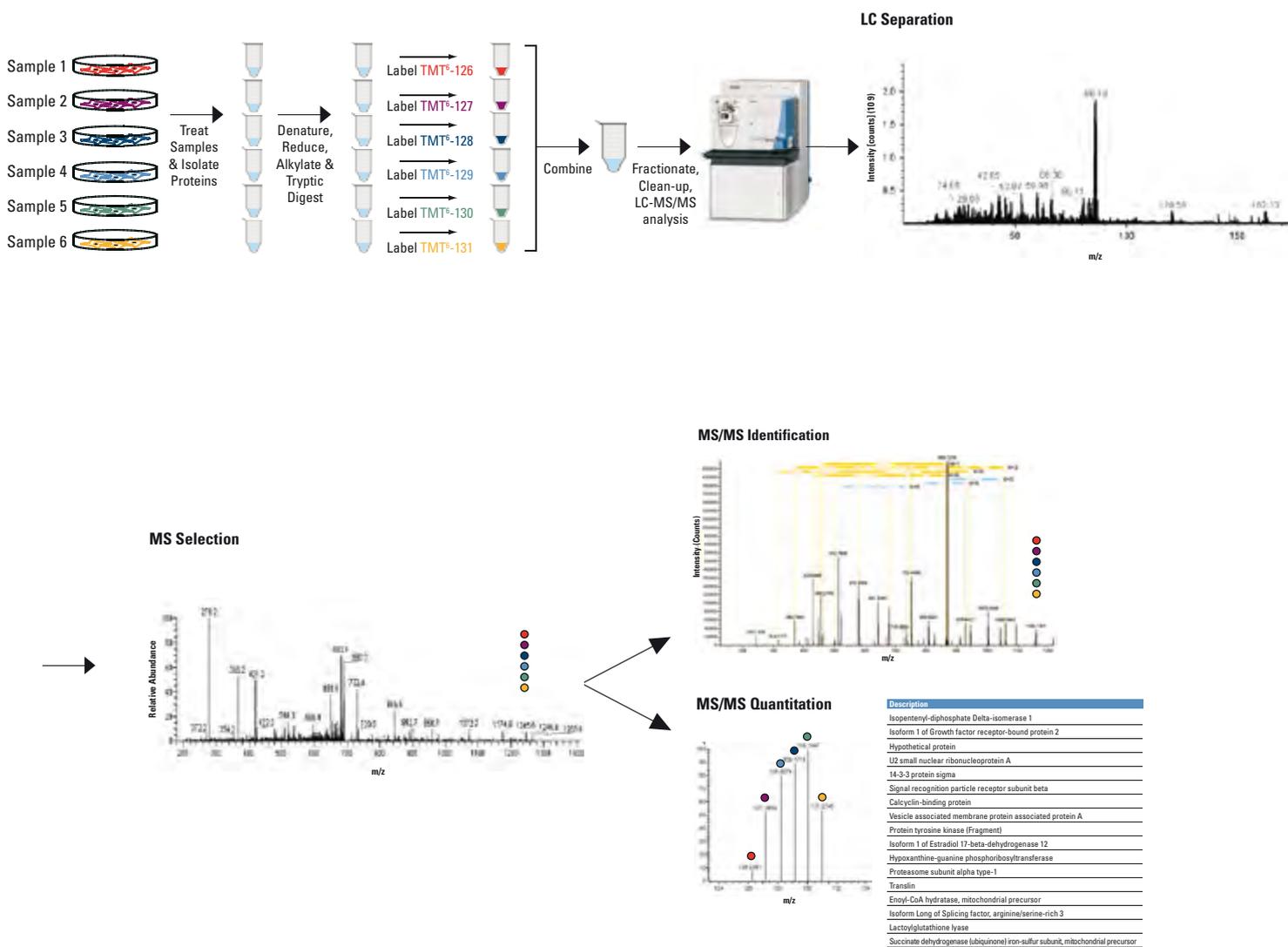Isobaric mass tagging kits for quantitative protein expression analysis

LC Separation

MS Selection

MS/MS Identification

MS/MS Quantitation

Description
Isopentenyl-diphosphate Delta-isomerase 1
Isoform 1 of Growth factor receptor-bound protein 2
Hypothetical protein
U2 small nuclear ribonucleoprotein A
14-3-3 protein sigma
Signal recognition particle receptor subunit beta
Calcyclin-binding protein
Vesicle associated membrane protein associated protein A
Protein tyrosine kinase (Fragment)
Isoform 1 of Estradiol 17-beta-dehydrogenase 12
Hypoxanthine-guanine phosphoribosyltransferase
Proteasome subunit alpha type-1
Translin
Enoyl-CoA hydratase, mitochondrial precursor
Isoform Long of Splicing factor, arginine/serine-rich 3
Lactoylglutathione lyase
Succinate dehydrogenase (ubiquinone) iron-sulfur subunit, mitochondrial precursor

Figure 2. Protein profiling with Thermo Scientific Tandem Mass Tags. Proteins from up to six treated samples are: 1. denatured; 2. digested with trypsin; 3. labeled with TMTsixplex tags; 4. combined; 5. cleaned or fractionated by strong cation exchange; 6. chromatographically separated, isolated and fragmented as peptides by in-line reversed phase LC-MS/MS; and 7. identified and quantified with Thermo Scientific BioWorks, Discoverer or Matrix Science Mascot Search Engine.
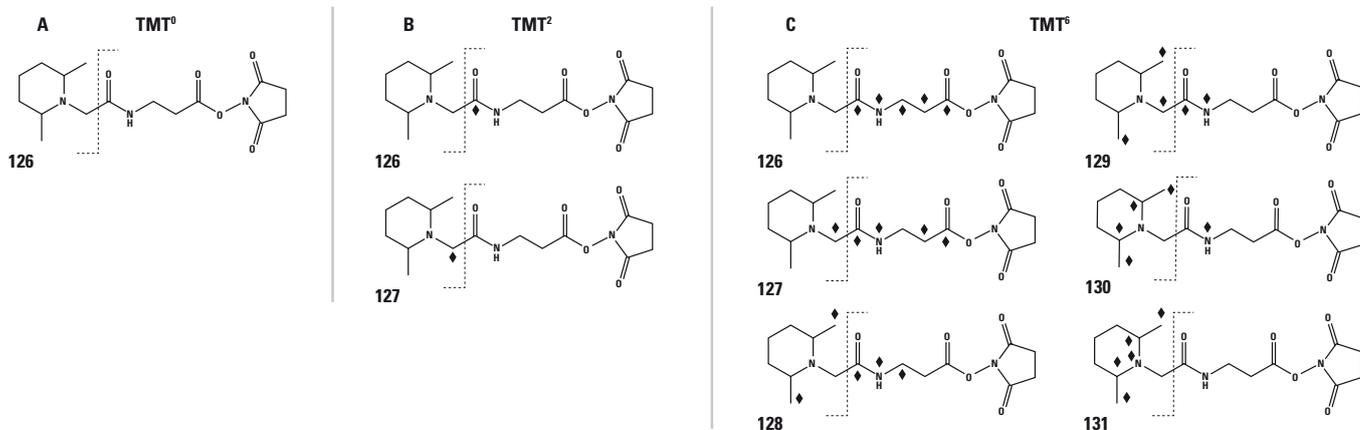
Figure 3. The Thermo Scientific TMT family of isobaric tag reagents. (A) TMTzero Reagent has no isotopic substitutions and is used for method development. (B) A pair of isobaric mass labels with a single isotopic substitution per tag is used for simple pairwise comparisons of relative protein expression. (C) A sixplex of isobaric mass labels each with five isotopic substitutions per tag is used. Used for complex analyses including multiplex patient screening, time-course analysis or dose escalation studies.
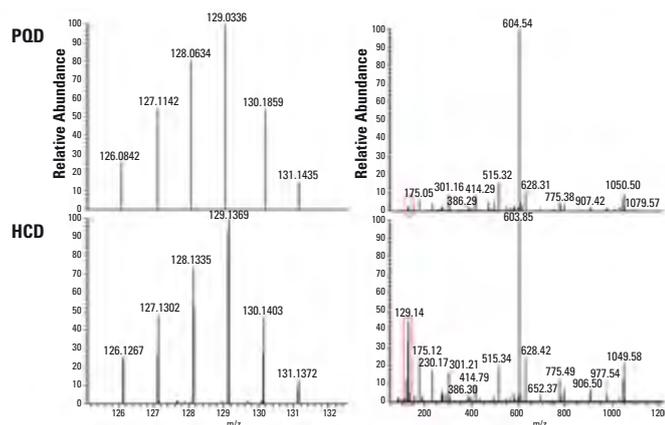
Figure 4. Analysis of Thermo Scientific TMTsixplex-labeled peptide by Pulsed Q Dissociation (PQD) and High Energy Collision Dissociation (HCD). TMTsixplex-labeled A'ITIFQER (2+) from rabbit glyceraldehyde-3-phosphate dehydrogenase in a 10-protein sample were mixed at a 1:2:3:4:2:0.5 ratio. Shown are the MS/MS spectra for the peptide fragment and reporter ion regions. PQD fragmentation was performed on a LTQ XL Ion Trap and by HCD on a high-resolution Thermo Scientific Orbitrap XL Mass Spectrometer.

Ordering Information

Product #	Description	Pkg. Size
90063	**TMTduplex Isobaric Mass Tagging Kit** Sufficient reagents for 5 duplex isobaric experiments with controls	15-rxn Kit
90060	**TMTduplex Isotopic Label Reagent Set, 5 x 0.8mg** Sufficient reagents for 5 duplex isotopic experiments with controls	10-rxn Set
90065	**TMTduplex Isobaric Label Reagent Set, 5 x 0.8mg** Sufficient reagents for 5 duplex isobaric experiments	10-rxn Set
90061	**TMTsixplex Label Reagent Set, 1 x 0.8mg** Sufficient reagents for 1 sixplex isobaric experiment	6-rxn Set
90062	**TMTsixplex Label Reagent Set, 2 x 0.8mg** Sufficient reagents for 2 sixplex isobaric experiments	12-rxn Set
90064	**TMTsixplex Isobaric Mass Tagging Kit** Sufficient reagents for 5 sixplex isobaric experiments with controls	35-rxn Kit
90066	**TMTsixplex Label Reagent Set, 5 x 0.8mg** Sufficient reagents for 5 sixplex isobaric experiments with controls	30-rxn Set
90068	**TMTsixplex Label Reagent Set, 2 x 5mg** Sufficient reagents for 12 sixplex isobaric experiments with controls	72-rxn Set
90067	**TMTzero Label Reagent** TMT0 Label Reagent, 5 x 0.8mg Sufficient reagent for 5 control experiments	5 x 0.8mg

References:

1 Thompson, A., *et al.* (2003). Tandem Mass Tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Anal. Chem.* **75(8):**, 1895-1904.
2. Dayon, L., *et al.* (2008). Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal. Chem.* **80(8):**, 2921-2931.

high-content analysis

Measures cytoskeletal structure and other key parameters of cell morphology

Kits for Cell Morphology, Cytoskeleton, Neuronal Function and Phenotypic Changes

The maintenance and control of cell shape, cell movement and cytokinesis and the organization of organelles are some of the most basic functions of the cell. Because changes in these features are often the consequence of cellular differentiation, cellular toxicity, pathology or other critical cellular event, their measurement against potential therapeutic targets can be critical. For certain cell types such as neurons, cellular morphological changes during development are necessary for the proper function in the tissue.

Such phenotypic changes can be monitored using the range of Thermo Scientific Cellomics High-Content Screening (HCS) Reagents designed for these functions, including kits for cytoskeletal rearrangement and cell motility, as well as kits for neurotoxicity that detect synapse formation and changes in neuronal morphology. In addition, these reagents with HCS or fluorescence microscopy provide crucial spatial information that other methods do not.

Application: Neurite Morphology and Synaptogenesis to Monitor Neurotoxicity

The synapse consists of two main structures: the presynaptic compartment that releases neurotransmitters and the postsynaptic compartment where these neurotransmission signals are received. Synaptic damage has often been recognized as the first sign of neurodegeneration in many different pathological conditions, including traumatic nerve injury, ischemic stroke, and neurodegenerative disorders such as Motor Neuron Disease, Alzheimer's, Parkinson's and Huntington's diseases as many synaptic proteins play an important role in the progression of neurodegenerative diseases. Since modulation of neurite and synaptic structures in neurons is closely related to the pathological process of neurological diseases or in neurodevelopment, synapse number in neurons can be a sensitive indicator for the change of neuronal function.

The Thermo Scientific Cellomics Synaptogenesis HCS Reagent Kit provides a new way of measurement for synaptic function utilizing full benefit of a sophisticated new technology in high content cell-based image analysis. The Synaptogenesis Kit consists of a nuclear stain to identify all cells, immunofluorescence against MAP2 to identify neurons, synaptoplysm as a pre-synaptic marker, and PSD95 as a post-synaptic marker. Co-localized synaptoplysm and PSD95 are probable locations of synapses. Using this technology and assay method, we could identify synaptic change over time and measure synaptic parameters as well as many neurite outgrowth and morphology measurements, which precede neurotoxicity in general, in an automated manner over different compound concentrations and conditions.

A major focus for drug discovery is identifying compounds that affect the growth of neurites. Drugs that promote nerve growth would aid treatment in a
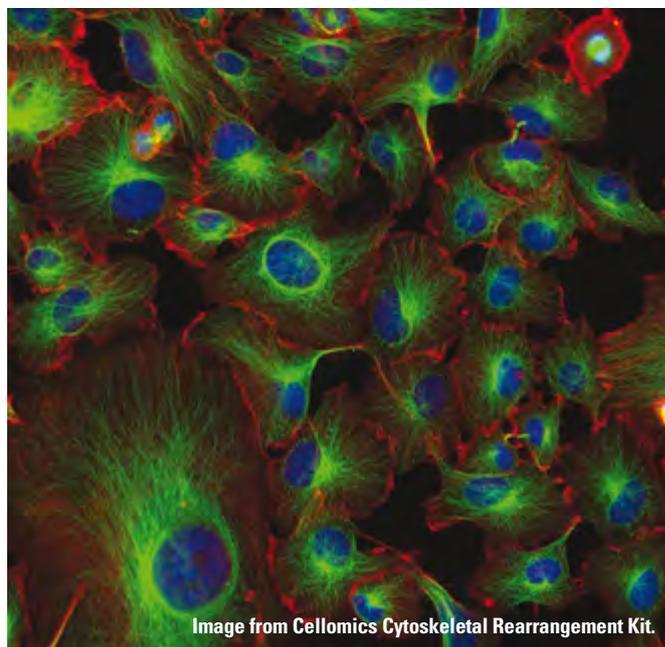

Image from Cellomics Cytoskeletal Rearrangement Kit.

wide variety of diseases and traumas that result in neuropathy and nerve injury. Examples of areas that would benefit from neuronal regeneration include spinal cord injuries, neuropathy resulting from diseases such as diabetes, stroke, and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.

The Thermo Scientific Cellomics Neurite Outgrowth HCS Reagent Kit enables quantification of neurons, neuronal like cells, and neurite outgrowth from a heterogeneous population of live cells growing on standard high-density microplates. The cells can either be neuronal-like cell models, such as PC12 or Neuroscreen*-1 Cells, or primary brain cultures that contain a subpopulation of neurons. The kit includes a primary antibody specific for neuron cell bodies and neurites and DyLight 488-conjugated Secondary Antibody. Cell nuclei are identified by DNA-specific Hoechst Dye.

The Neurite Outgrowth Kit reagents, in combination with the Thermo Scientific ArrayScan VTI HCS Reader and the (Extended) Neurite Outgrowth BioApplication software enable automated plate handling, focusing, cell image acquisition, analysis, and neurite outgrowth quantification.

For a more detailed description of the image processing algorithm, see the Neurite Outgrowth or Extended Neurite Outgrowth BioApplication Guide that accompanies the software.
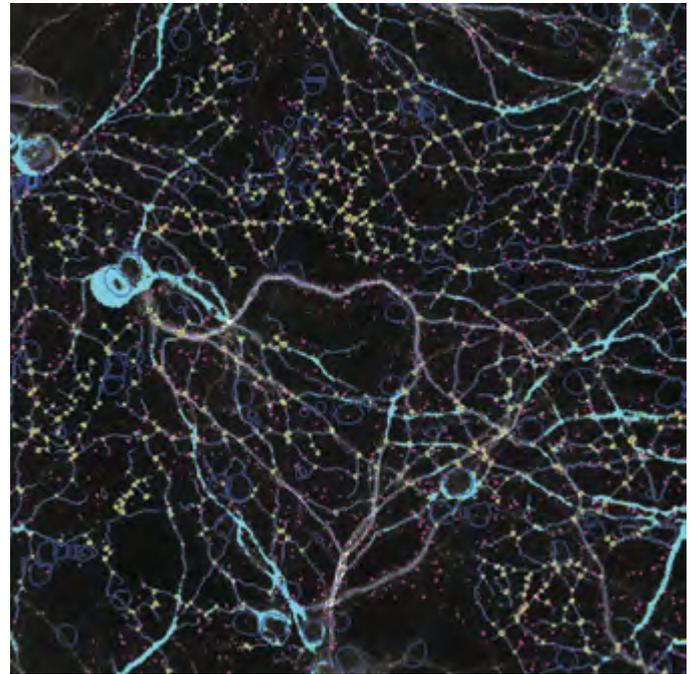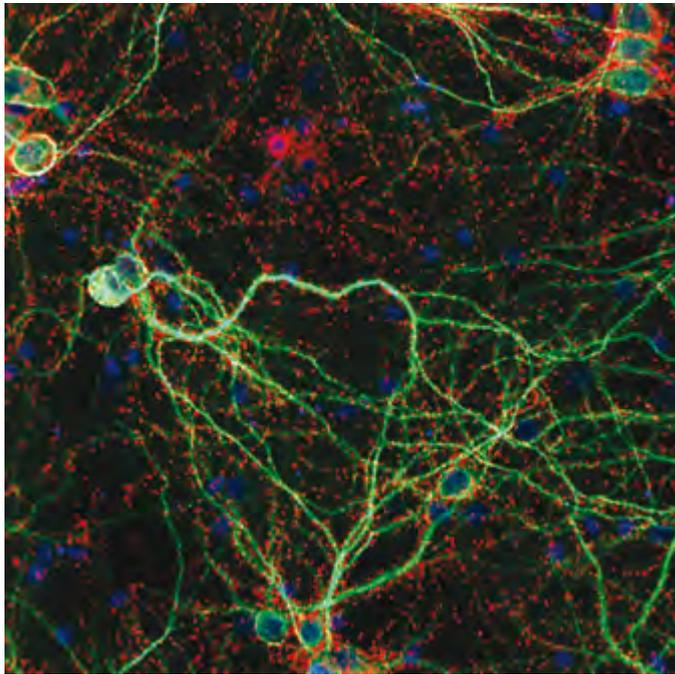
Raw Image
Presynaptic Marker Synaptophysin (red)
Neuronal Marker MAP2 (green)

Analyzed Image
Branch Point (white)
Localized Synaptophysin (purple)
Neuronal Trace (blue)

Automated measurement of presynaptic vesicles and neurites using Thermo Scientific Cellomics Neuronal Profiling v3.5 Bio Application Software Molecule. Mouse cortical neurons (14 DIV) were stained for synaptophysin (red) and MAP2 (green) (left panel), and analyzed (right panel) with the ArrayScan* VTI HCS Reader and the Neuronal Profiling v3.5 BioApplication.
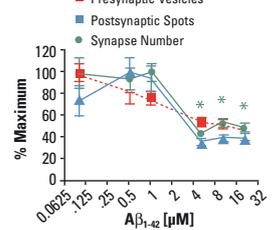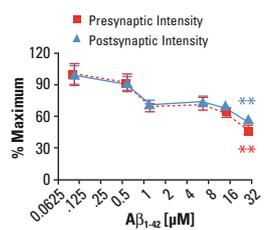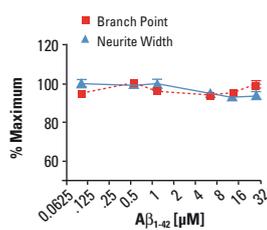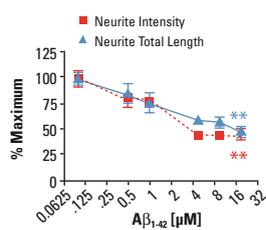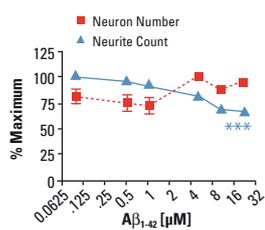
Neurite and synapse changes in response to Aβ_{1-42} aggregates. Rat hippocampal primary neurons were cultured for 50 days. Dose-dependent responses of Aβ_{1-42} aggregates were investigated. 500 μM Aβ_{1-42} was incubated at 37°C in media for three days to induce oligomerization. Neurons were incubated with the Aβ_{1-42} oligomers for 48 hours, and then fixed, stained and analyzed. Aβ_{1-42} toxicity leads to synapse loss. (Student's t-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

high-content analysis

Measures cytoskeletal structure and other key parameters of cell morphology

Neurite Intensity

Neurite Width

Neurite Number

Neurite Length

Branch Point

Synaptic Vesicles
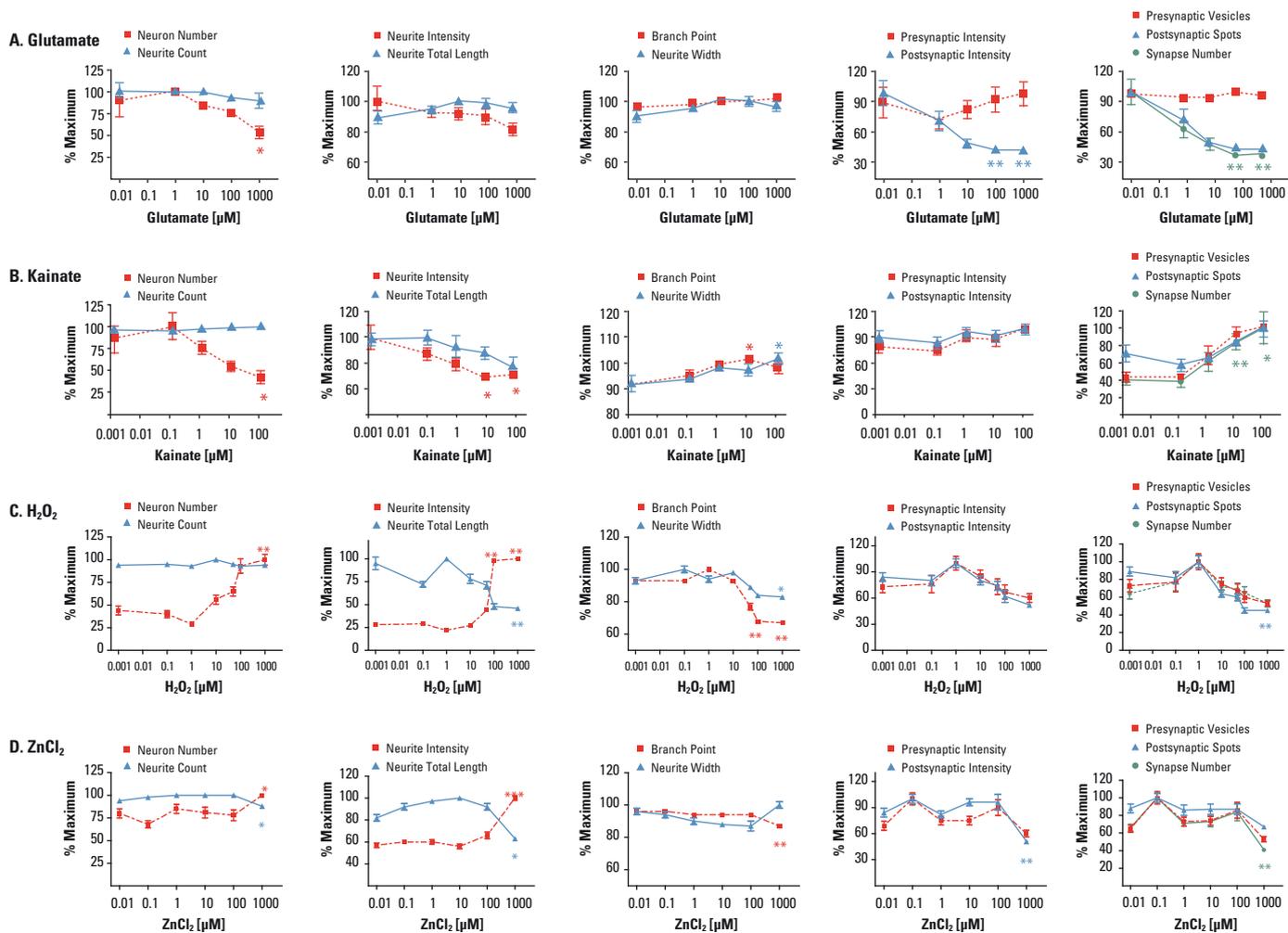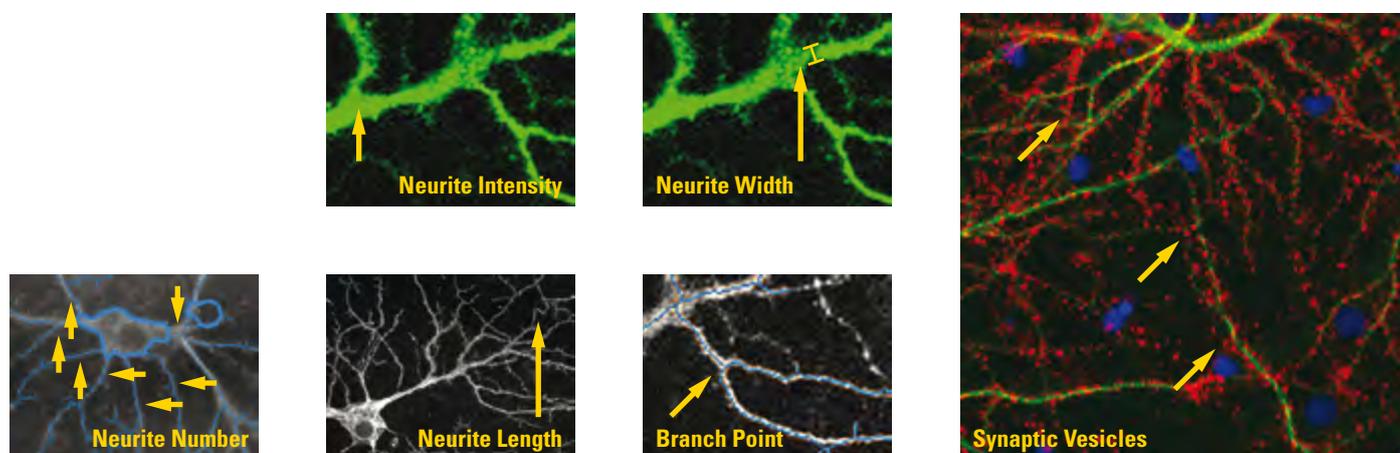
Automated measurements and correlation of neuronal morphology and synapses over different compound doses. Mouse, rat cortical or hippocampal primary neurons were cultured for 21 days, and the dose-dependent responses of drugs towards various properties of these neurons were investigated. (**A**) Glutamate with 10 mM glycine in HBSS was treated for 30 minutes, washed and replaced with culture media. After 24-hour incubation, neurons were fixed, stained and analyzed. (**B**) Kainate, (**C**) H_2O_2, and (**D**) Zinc were treated for 24 hours in culture media. (Student's t-test, *p<0.05, **p<0.01, ***p<0.001).

For more convenience and flexibility you can compose your kit by ordering the stand-alone components.

Ordering Information

Discontinued Kits

Product #	Description	Pkg. Size
8402401	**Cytoskeletal Rearrangement : F-actin, Tubulin and Whole Cell Probes**	(1 x 96 well plates)
8402402	**Cytoskeletal Rearrangement : F-actin, Tubulin and Whole Cell Probes**	(5 x 96 well plates)

Stand-alone components

Product #	Description	Pkg. Size
MA1-19187	**Pierce beta-3 Tubulin Monoclonal Antibody (TU-20)**	100µg
84545	**Pierce Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 650 conjugate**	1mL
21834	**Pierce Phalloidin, DyLight 554 conjugate**	300 units
62248	**Hoechst Dye**	30µL
840350	**Whole Cell Stain Green**	
8423000	**Buffer Kit #3, including:** Wash Buffer 10X (100mL) Permeabilization buffer 10X (100mL) Blocking Buffer 10X (85mL)DAPI Dye (30µL)	Kit

Discontinued Kits

Product #	Description	Pkg. Size
K0700011	**Neurite Outgrowth Kit**	(5 x 96 well plates)
R0105131	**Neurite Outgrowth Kit**	(50 x 96 well plates)

Stand-alone components

Product #	Description	Pkg. Size
MA1-19187	**Pierce beta-3 Tubulin Monoclonal Antibody (TU-20)**	100µg
35502	**Pierce Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 488 conjugate**	1mL
8421000	**Buffer Kit #1, including:** Wash Buffer 10X (100mL) Permeabilization buffer 10X (100mL) Blocking Buffer 10X (85mL) Hoechst Dye (30µL)	Kit
8406900	**Neurite Outgrowth Buffer** 10X proprietary formulation	100mL

Discontinued Kits

Product #	Description	Pkg. Size
8408402	**Synaptogenesis Kit**	(5 x 96 well plates)
8408403	**Synaptogenesis Kit**	(50 x 96 well plates)

Stand-alone components

Product #	Description	Pkg. Size
PA1-1043	**Pierce Synaptophysin Polyclonal Antibody**	100µg
MA1-046	**Pierce PSD95 Monoclonal Antibody (7E3-1B8)**	100µL
MA5-12826	**Pierce MAP 2abc Monoclonal Antibody (AP18)**	500µL
84546	**Pierce Goat anti-Rabbit IgG (H+L) Secondary Antibody, DyLight 650 conjugate**	1mL
35502	**Pierce Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 488 conjugate**	1mL
84540	**Pierce Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 550 conjugate**	1mL
8423000	**Buffer Kit #3, including:** Wash Buffer 10X (100mL) Permeabilization buffer 10X (100mL) Blocking Buffer 10X (85mL)DAPI Dye (30µL)	Kit

high-content analysis

Analysis of neuronal differentiation from stem cells

Michael Anhalt; Douglas E. Hughes, Ph.D.; and Suk J. Hong, Ph.D.; Thermo Fisher Scientific

High-content analysis (HCA) based on automated image acquisition, image processing and analysis, and bioinformatics has a power to enhance discovery research using stem cells. Human embryonic stem cells (hESCs) are difficult to study because of the specialized conditions that affect growth rate, death and differentiation. HCA with optimized cell culture conditions provides a valuable tool for stem cell research. HCA will improve productivity and consistency in research.

The Thermo Scientific Cellomics Neurite Outgrowth Reagent enables neurons, neuronal-like cells and neurite outgrowth quantification from a heterogeneous population of live cells growing on standard high-density microplates (Figures 1 and 2). Undifferentiated cells were differentiated to neuronal cells and then were fixed, permeabilized and probed with dyes and antibodies. Hoechst 33342 dye is used to stain the nucleus, identifying every cell within a population. The kit includes a neuron-specific antibody and a highly stable fluorescent dye (DyLight 488 Dye) conjugated to a secondary antibody. Cells prepared with the Neurite Outgrowth Kit can be imaged and analyzed using the ArrayScan VTI HCS Reader or visualized directly via standard fluorescence microscopy.

Highlights:

- **Specific** – neuron-specific fluorescent staining identifies neuronal cell bodies and neurites in a heterogeneous cell population without isolation steps
- **Versatile** – reagents can be used on primary cells and neuron-like cells growing on standard high-density microplates
- **Validated** – the optimized protocol requires approximately 2.5 hours post-compound treatment to complete post-compound treatment to complete
- **Compatible** – plates can be read with automated systems such as the ArrayScan VTI HCS Reader
- **Easy to use** – cells can be prepared on slides for standard fluorescence microscopic analysis
- **Safe and quick** – no radioactivity, cell lysis, purification or filtration steps
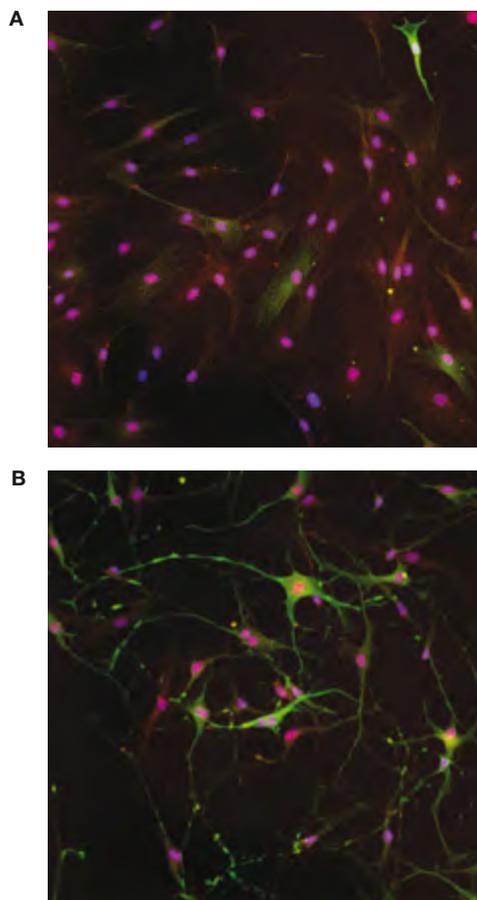
Figure 1. Detection of neuronal differentiation from human mesenchymal stem cells. Non-differentiated human mesenchymal stem cells **(Panel A)** and cells differentiated in differentiation media for 48 hours **(Panel B)** were stained with the Cellomics Neurite Outgrowth Kit and Whole Cell Stain Orange.
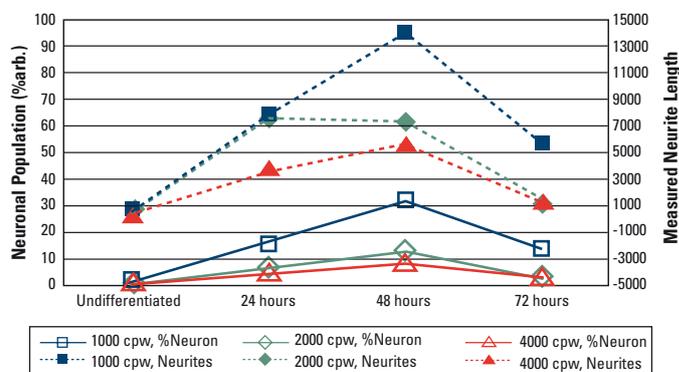
Figure 2. High-content analysis of neurons differentiated from human mesenchymal stem cells. Stem cells were plated at three densities (1,000, 2,000 or 4,000 cells per well) on 96-well microplates and differentiation was induced for 24 hours, 48 hours or 72 hours, respectively. Cells were stained with the Cellomics Neurite Outgrowth Kit and analyzed with Cellomics Neuronal Profiling V3.5 Bioapplication. Two of the output analysis features, neuronal population and neurite length, were graphed. Cell density at 1,000 cells per well (cpw) and differentiation at 48 hours resulted in good neuronal differentiation.

primary antibodies

Dedicated antibodies to visualize and detect neurobiology events

We offer antibodies against key proteins and many other components involved in neurobiology.
Go to www.thermoscientific.com/pierce-antibodies and discover our complete portfolio of immunoreagents.
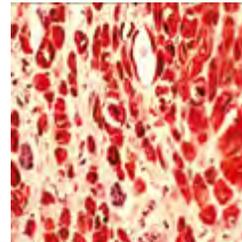
Custom antibodies for novel protein targets are also available with antibody-on-demand custom antibody production services. For more information, visit www.pierce-antibodies.com/custom-antibodies.
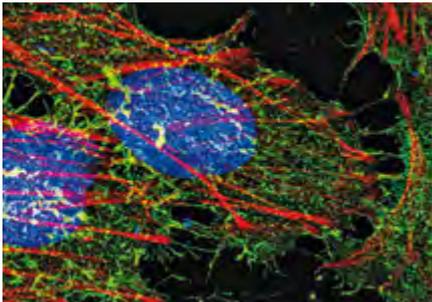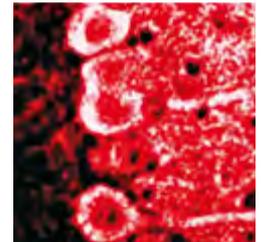

CD44 Mouse Monoclonal (E.649.3) #MA5-14983
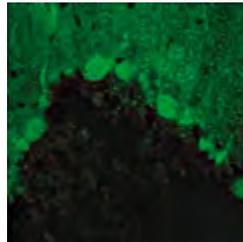
PSD93 Rabbit Polyclonal #PA1-043

S100 A4 Rabbit Polyclonal #PA5-16586

Calmodulin Mouse Monoclonal (2D1) #MA3-917
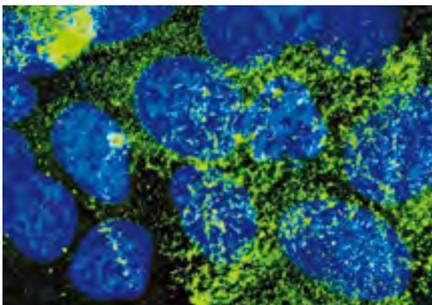
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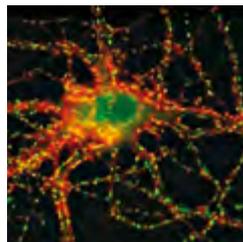
SynGAP Rabbit Polyclonal #PA1-046
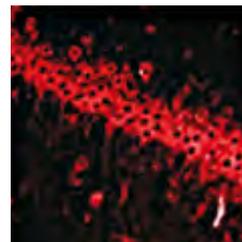
NMDA Receptor 1 Rabbit Polyclonal #PA3-102
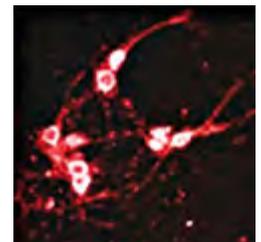
NMDA Receptor 2B Rabbit Polyclonal #PA3-104

Ordering Information for Select Thermo Scientific Pierce Antibodies

| Product #	Description	Target Species	Applications	Pkg. Size
MA3-042	Acetylcholinesterase Monoclonal Antibody (HR2)	Hu, Rb, Bv, Fe	ELISA, IHC (F), IP	200µL
MA3-041	Acetylcholinesterase Monoclonal Antibody (ZR3)	Fe, GP, Rb, Rt	IHC, IP	100µL
PA1-37077	Amyloid β Polyclonal Antibody	Hu	IHC (P)	1mL
PA1-749	Ataxin 7 Polyclonal Antibody	Dm, Hu	IHC (F), WB	100µg
MN1150	β Amyloid Monoclonal Antibody (10H3)	Hu	ELISA, IHC, Neu, WB	100µg
PA1-38703	β Synuclein Polyclonal Antibody	Hu, Rt	IHC (P)	1mL
MA3-917	Calmodulin Monoclonal Antibody (2D1)	Ba, Bv, Ck, Rt	ELISA, ICC, WB	200µL
MA3-944	Calpastatin Monoclonal Antibody (1F7E3D10)	Bv, Hu, Po, Rt	ICC, WB	100µL
PA1-745	Cannabinoid Receptor I Polyclonal Antibody	Hu, Ms, Nhp, Rt	FACS, ICC, IHC (P), WB	100µL
PA1-744	Cannabinoid Receptor II Polyclonal Antibody	Hu, Rt	FACS, ICC, IF, IHC (P, F), WB	100µL
MA5-14983	CD44 Monoclonal Antibody (E.649.3)	Hu	FACS, ICC, IHC (P), IP, WB	100µL
MA5-15114	CREB Monoclonal Antibody (C.12.2)	Hu, Ms, Nhp, Rt	FACS, ICC, IF, WB	100µL
MA1-083	CREB Monoclonal Antibody (LB9)	Hu, Ms, Rt	ELISA, WB	100µg
MA5-11768	Cystic Fibrosis Transmembrane Regulator Monoclonal Antibody (M3A7)	Hu, Ms, Rt	IF, IP, WB	500µL
MA3-921	Dihydropyridine Receptor a 2 Monoclonal Antibody (20A)	GP, Hu, Ms, Rb, Rt	ICC, IF, IHC (P, F), WB	100µL
PA1-46163	DISC1 Polyclonal Antibody	Hu, Ms, Rt	IHC (P)	100µL

For species and application abbreviations, see page 33.
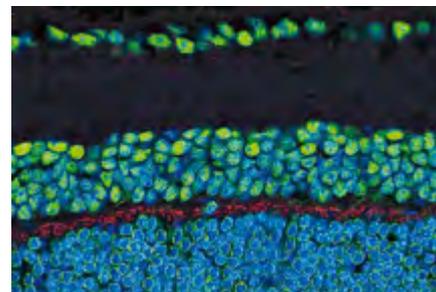
primary antibodies

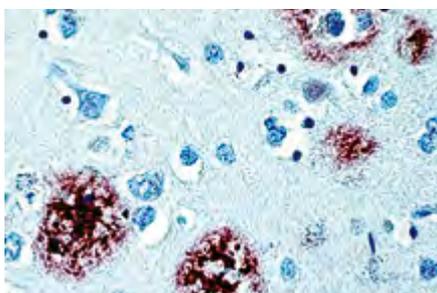Dedicated antibodies to visualize and detect neurobiology events
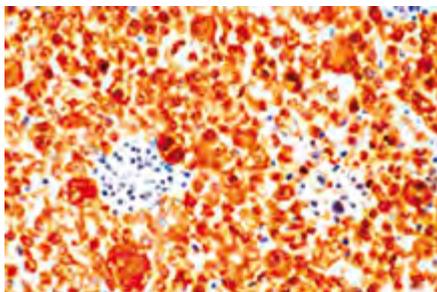
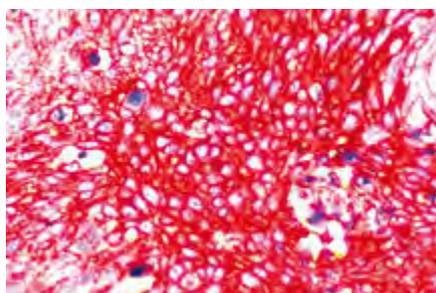S100 Protein Mouse Monoclonal (4C4.9)
#MA5-12969
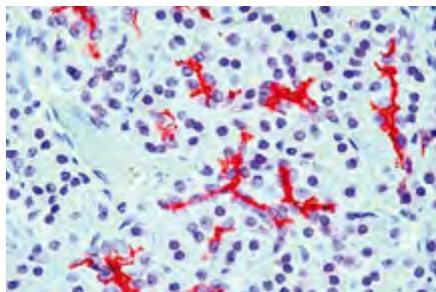
Cystic Fibrosis Transmembrane Regulator Mouse
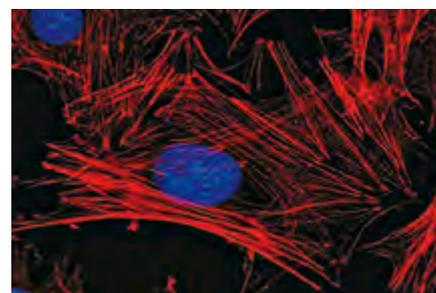Monoclonal (M3A7) #MA5-11768

Phospho-NMDAR1 Rabbit Polyclonal (Ser890) #PA5-17750

Amyloid β Rabbit Polyclonal #PA1-37077

GLUT-1 Mouse Monoclonal (SPM498)
#MA5-11315

Phospho-GSK-3β Rabbit Monoclonal (Ser9) (C.367.3)
#MA5-14873

Ordering Information for Select Thermo Scientific Pierce Antibodies

| Product #	Description	Target Species	Applications	Pkg. Size
PA3-925	Dopamine β Hydroxylase Polyclonal Antibody	Hu, Ms, Rt	ICC, IF, IHC (F), WB	100µL
PA5-17014	Enolase-2 Polyclonal Antibody Rabbit	Hu, Ms	WB	100µL
MA5-14843	FGF Receptor 3 Monoclonal Antibody (T.994.9)	Hu	ICC, IHC (P), IP, WB	100µL
MN1180	Glial Fibrillary Monoclonal Antibody (MIG-G2)	Hu	IHC staining	500µL
MA5-12023	Glial Fibrillary Acidic Protein Monoclonal Antibody (ASTRO6)	Hu, Rt, Ck, Po	IF, IHC (P), WB	500µL
MA5-11315	GLUT-1 Mouse Monoclonal (SPM498)	Hu, Rt	IHC (P)	500µL
PA1-036	iNOS Polyclonal Antibody	Hu, Ms, Rt	IF, WB	200µL
MA1-06100	Laminin Monoclonal Antibody (A5)	Hu, Ms	ICC, IHC (F)	100µg
MA1-074	LAP1 Monoclonal Antibody (RL13)	Rt	IF, IP, WB	200µL
PA1-742	Munc18 Polyclonal Antibody	Ms, Rt	IHC (F), IP, WB	100µg
MA1-085	Neurofibromin Monoclonal Antibody (McNFn27b)	Hu, Ms, Rt	IHC, WB	200µg
MN1190	Neurofilament Monoclonal Antibody (MIG-N18)	Hu	IHC	500µL
MA1-2012	Neurofilament, Heavy Chain Monoclonal Antibody (3G3)	Hu, Rt	IF, IHC, WB	100µg
MA1-2010	Neurofilament, Light Chain Monoclonal Antibody (DA2)	Hu, Rt	ELISA, ICC, IF, WB	100µg
MA1-2011	Neurofilament, Medium Chain Monoclonal Antibody (3H11)	Hu, Rt	IF, IP, WB	100µg
PA3-212	Neuropeptide S (NPS) Polyclonal Antibody	Hu , Ms , Rt	ICC, IHC (P)	100µL
PA3-214	Neurotensin Receptor 1 (NTSR1) Polyclonal Antibody	Hu	ICC, WB	100µL
PA5-17045	NHERF1 Polyclonal Antibody	Hu	ICC, WB	100µL
PA3-102	NMDA Receptor 1 Polyclonal Antibody	Hu, Ms, Rt	ELISA, ICC, IHC, IP, WB	200µL
PA3-103	NMDA Receptor 1 Polyclonal Antibody	Hu, Ms, Rt	ELISA, ICC, IHC, IP, WB	200µL
MA1-2014	NMDA Receptor 2B Monoclonal Antibody (NR2B)	Hu, Ms, Rt	ICC, WB	100µg
PA3-104	NMDA Receptor 2B Polyclonal Antibody	Hu, Ms, Rt	ELISA, ICC, IHC, IP, WB	200µL
PA3-032A	nNOS Polyclonal Antibody	Bv, Ms, Rb, Rt	IHC (F), IP, WB	100µL
PA1-751	Parkin Polyclonal Antibody	Hu, Rt	IHC, WB	100µg
PA1-38412	Parkin Polyclonal Antibody	Hu, Ms, Rt	IHC (P)	1mL
PA1-933	Parvalbumin Polyclonal Antibody	Hu, Rt	ELISA, IHC (P), IP, WB	100µg
MN1020	PHF-tau (Ser202/Thr205)a Monoclonal Antibody (AT8)	Hu	ELISA, IF, IHC(P), WB	100µg

For species and application abbreviations, see page 33.
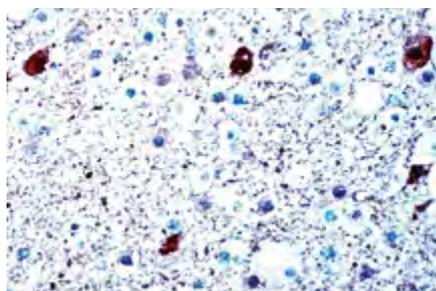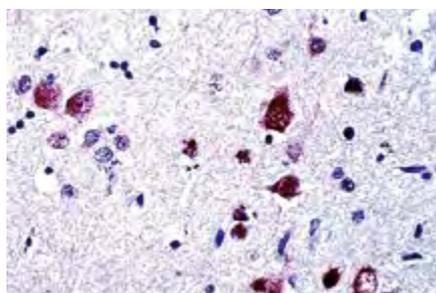
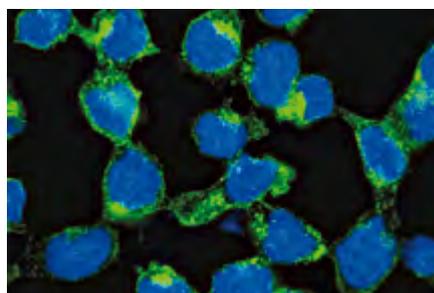β Synuclein Rabbit Polyclonal #PA1-38703
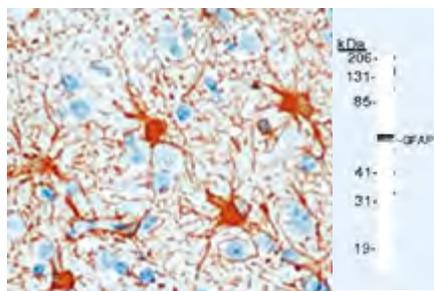
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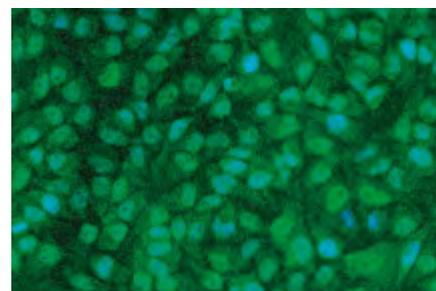
CREB Mouse Monoclonal (LB9) #MA1-083

Parkin Rabbit Polyclonal #PA1-38412

FGF Receptor 3 Rabbit Monoclonal (T.994.9) #MA5-14843

Guide to Abbreviations

Target Species Abbreviations
Am amphibian
Ba bacteria
Bv bovine
Ca canine
Ck chicken
Dm Drosophila
Eq equine
Fe feline
Fs fish
GP guinea pig
Hu human
Ms murine
Nhp non-human primate
Po porcine
Rb rabbit
Rt rat
Xl Xenopus laevis

Validated Applications Abbreviations
DB Dot blot
ELISA ELISA
FACS Flow cytometry
ICC Immunocytochemistry
IF Immunofluorescence
IHC Immunohistochemistry
IHC(F) Immunohistochemistry (Frozen)
IHC(P) Immunohistochemistry (Paraffin)
IP Immunoprecipitation
Neu Neutralization
WB Western blot

Ordering Information for Select Thermo Scientific Pierce Antibodies

Product #	Description	Target Species	Applications	Pkg. Size
MN1040	PHF-tau (Thr231) Monoclonal Antibody (AT180)	Hu	IF, IHC, WB	100µg
MA5-14873	Phospho-GSK-3β (Ser9) Monoclonal Antibody (C.367.3)	Hu, Ms, Nhp, Rt	ICC, IHC (P), WB	100µL
PA5-17750	Phospho-NMDAR1 (Ser890) Polyclonal Antibody	Hu, Ms, Rt	IF, WB	100µL
PA1-043	PSD93 Polyclonal Antibody	Rt	IHC (F), WB	100µg
MA1-045	PSD95 Monoclonal Antibody (6G6-1C9)	Ms, Rt	ICC, IF, IP, WB	100µL
MA1-046	PSD95 Monoclonal Antibody (7E3-1B8)	Ms, Rt, Xl	ICC, IF, IHC (F), IP, WB	100µL
PA1-075	RAGE Polyclonal Antibody	Ms, Rt	IHC (F), WB	100µg
MA3-916	Ryanodine Receptor Monclonal Antibody (C3-33)	Hu, Ms, Rt, Rb, Am, Ca, Ck, Fs, GP	FACS, ICC, IF, IHC (F), IP, WB	100µg
PA5-16586	S100 A4 Polyclonal Antibody	Hu, Ms, Rt, Bv, Ca, Eq	IHC (P)	500µL
MA5-12969	S100 Protein Monoclonal Antibody (4C4.9)	Hu, Ms, Rt, Bv	IHC (P)	500µL
MN1280	SNAP25 Monoclonal Antibody (11D2)	Hu	ELISA, IHC, WB	100µg
MA5-11157	Superoxide Dismutase 2 Monoclonal Antibody (37CT127.5.11.6)	Hu	IHC, WB	100µg
MN1260	Synapsin Monoclonal Antibody (5C8)	Hu, Po	ELISA, IHC, WB	100µg
OSS00021W	Synaptophysin Polyclonal Antibody	Ms, Rt	IHC, WB	100µL
PA1-046	SynGAP Polyclonal Antibody	Ms, Rt	ICC, WB	100µg
MA1-745	Syntrophin Monoclonal Antibody (1351)	Am, Ca, Ck, Hu, Ms, Rt	IF, IP, WB	100µg
MN1290	Synuclein Monoclonal Antibody (9B6)	Hu	ELISA, IHC, WB	100µg
MN1010	Tau Monoclonal Antibody (BT2)	Bv, Hu, Nhp, Rt	ELISA, WB	100µg
MN1000	Tau Monoclonal Antibody (HT7)	Bv, Hu	ELISA, IHC, WB	100µg
PN1000	Tau Polyclonal Antibody	Bv, Hu, Rt	IHC, WB	500µg
PA1-777	TPH1 Polyclonal Antibody	Hu, Ms, Nhp, Rb, Rt	ICC, IP, WB	100µL
PA1-778	TPH2 Polyclonal Antibody	Hu, Ms, Nhp, Rb, Rt	ICC, IP, WB	100µL
PA1-748	Vanilloid Receptor 1 Polyclonal Antibody	Hu	IF, IHC (F)	100µg
MA5-15157	VEGF Receptor 2 Monoclonal Antibody (B.309.4)	Hu, Ms	FACS, ICC, IF, IHC (P), IP, WB	100µL

For species and application abbreviations, see page 33.

instrumentation and software

Thermo Scientific ArrayScan VTI HCS Reader

The ArrayScan VTI HCS Reader is a highly flexible, modular high content screening instrument designed for high-capacity automated fluorescence imaging and quantitative analysis of fixed and live cells. The instrument features Carl Zeiss* optics, broad spectrum white-light source, 12-bit cooled CCD camera, and integrated acquisition and analysis software.

The ArrayScan VTI HCS Reader has a scientific grade digital camera with multiple resolution modes. The system has an automated objective changer, allowing for control over optical resolution and field of view. It uses a high-intensity white light source delivering a broad (350-700 nm) illumination spectrum. The system includes a 10-position wheel for excitation filters and a five position turret for dichroic/emission cubes.

Modular Functionality

The ArrayScan VTI HCS Reader has a modular design that allows for easy system expansion for a variety of HCS applications. Each component was designed and tested to be fully integrated with the instrument's hardware and software. For a complete listing of modules and their functionality, visit www.thermoscientific.com/hcs.

Integrated Software

The ArrayScan VTI HCS Reader includes Thermo Scientific Cellomics iQ–High Content intelligent acquisition software and the proprietary auto-focus software Thermo Scientific Cellomics AccuFocus. Cellomics iQ can automate multiple protocols and automate changes to the objective and filter wheel, and its user-friendly menus are easy to navigate. Thermo Scientific High-Content Informatics (HCi*) automatically stores and organizes data in an enterprise-class database. Analysis and visualization are available offline, and images from third-party sources can be imported into the database for analysis and storage.

Thermo Scientific Cellomics BioApplications Image Analysis Software

Cellomics BioApplications provide scientists with sophisticated measurements that reflect the biological properties of the cell. The numerous features reported for each cell include size, shape, amount of fluorescent label, and pattern of fluorescence. Each BioApplication reports cellular data at the individual cell level as well as at the whole well and subpopulation level.

Highlights:
- Validated algorithms with optimized protocols for image acquisition and analysis
- Streamlined analysis suitable for both assay development and screening
- Comprehensive documentation and pre-made image analysis protocols
- License to BioApplications includes use on your vHCS*: Discovery Toolbox workstations
- Flexible bundling of BioApplications for cost-effective screening

The following modules are available:
- Cell Cycle
- Cell Health Profiling
- Cell Motility
- Cell Spreading
- Compartmental Analysis
- Cyto-Cell Membrane
- Translocation
- Cytoplasm-to-Nucleus
- Translocation
- Extended Neurite
- Outgrowth
- GPCR Signaling
- Micronucleus
- Molecular Translocation
- Morphology Explorer
- Multiparameter Cytotoxicity
- Neurite Outgrowth
- Neuronal Profiling
- Spot Detector
- Target Activation

Cell Lysis Products Technical Handbook Supplement

This 14 page handbook includes:
- neuronal protein extraction kits
- subcellular fractionation kits
- universal nuclease
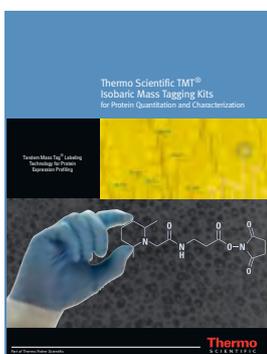- protease/phosphatase inhibitors

Reference number 1602377

GTPase Research Tools

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- global GTPase profiling

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Isobaric mass tagging kits

This 6 page brochure includes:
- an overview of our Thermo Scientific TMT Isobaric Mass Tagging Kits for Protein Quantitation and Characterization

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