#7087C Store at 4°C

PathScan® Phospho-TrkB (panTyr) Chemiluminescent Sandwich ELISA Kit

1 Kit
(96 assays)
Low volume microplate

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 05/24/19

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: The PathScan® Phospho-TrkB (panTyr) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tyrosine-phosphorylated TrkB protein with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. A TrkB Mouse mAb has been coated onto the microwells. After incubation with cell lysates, TrkB (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Biotinylated Phospho-Tyrosine Detection Antibody is added to detect captured tyrosine-phosphorylated TrkB protein. HRP-linked Streptavidin is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of tyrosine-phosphorylated TrkB protein.

*Antibodies in kit are custom formulations specific to kit.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
TrkB Mouse mAb Coated Microwells*	37793	96 tests		4°C
Phospho-Tyrosine Mouse Detection mAb (Biotinylated)	14055	1 each	Green (Lyophilized)	4°C
HRP-Linked Streptavidin (ELISA Formulated)	11805	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	5.5 ml	Green	4°C
HRP Diluent	13515	5.5 ml	Red	4°C
Luminol/Enhancer Solution	84850	3 ml		RT
Stable Peroxide Buffer	42552	3 ml		RT
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

Low volume microplate *12 8-well modules – Each module is designed to break apart for 8 tests.
Note: This kit contains components with mixed storage temperatures. Please store this entire kit at 4°C for long term storage. Upon first use, please store each component as indicated in the chart above and on individual component labels.

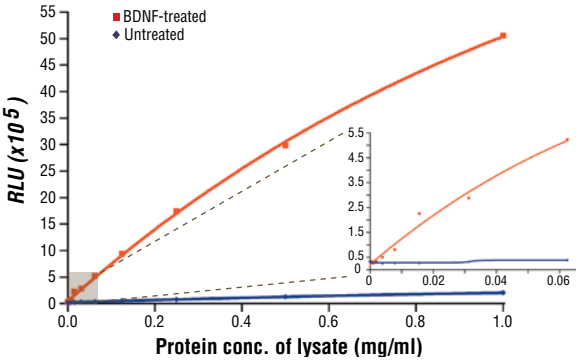

Figure 1. Relationship between protein concentration of lysates from untreated and BDNF-treated 3T3/TrkB cells and immediate light generation with chemiluminescent substrate is shown. After starvation, 3T3/TrkB cells (85% confluent) were treated with Human Brain-Derived Neurotrophic Factor (BDNF) #3897 (100 ng/ml, 2 min) at 37°C, and lysed with 1X CEll Lysis Buffer (10X) #9803. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

Background: The family of Trk receptor tyrosine kinases consists of TrkA, TrkB, and TrkC. While the sequence of these family members is highly conserved, they are activated by different neurotrophins: TrkA by NGF, TrkB by BDNF or NT4, and TrkC by NT3 (1). Neurotrophin signaling through these receptors regulates a number of physiological processes, such as cell survival, proliferation, neural development, and axon and dendrite growth and patterning (1). In the adult nervous system, the Trk receptors regulate synaptic strength and plasticity. TrkA regulates proliferation and is important for development and maturation of the nervous system (2). Phosphorylation at Tyr490 is required for Shc association and activation of the Ras-MAP kinase cascade (3,4). Residues Tyr674/675 lie within the catalytic domain, and phosphorylation at these sites reflects TrkA kinase activity (3-6). Point mutations, deletions, and chromosomal rearrangements (chimeras) cause ligand-independent receptor dimerization and activation of TrkA (7-10). TrkA is activated in many malignancies including breast, ovarian, prostate, and thyroid carcinomas (8-13). Research studies suggest that expression of TrkA in neuroblastomas may be a good prognostic marker as TrkA signals growth arrest and differentiation of cells originating from the neural crest (10).

Many tyrosine phosphorylation sites are conserved between TrkA and TrkB: Tyr490 of TrkA corresponds to Tyr512 in TrkB, and Tyr674/675 of TrkA to Tyr706/707 in TrkB of the human sequence (14). TrkB is overexpressed in tumors such as neuroblastoma, prostate adenocarcinoma, and pancreatic ductal adenocarcinoma (15). Research studies have shown that in neuroblastomas, overexpression of TrkB correlates with an unfavorable disease outcome when autocrine loops signaling tumor survival are potentiated by additional overexpression of brain-derived neurotrophic factor (BDNF) (16-18). An alternatively spliced truncated TrkB isoform lacking the kinase domain is overexpressed in Wilms' tumors and this isoform may act as a dominant-negative regulator of TrkB signaling (17).

Specificity/Sensitivity: PathScan® Phospho-TrkB (panTyr) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of TrkB protein when phosphorylated at Tyr residues in human cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Chemiluminescent Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

NOTE: Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 0.5 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 0.5 ml volume of reconstituted Detection Antibody to 5.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 0.5 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (5.5 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
6. **Sample Diluent:** Blue colored diluent for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **Luminol/Enhancer Solution** and **Stable Peroxide Buffer**

**Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

B Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 50 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 150 µl each time for each well.
   c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   d. Clean the underside of all wells with a lint-free tissue.
5. Add 50 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 µl of reconstituted HRP-linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the Detection Reagent Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. *Optimal signal intensity is achieved when read within 10 min.*

Background References:

(1) Huang, E.J. and Reichardt, L.F. (2003) *Annu Rev Biochem* 72, 609-42.

(2) Segal, R.A. and Greenberg, M.E. (1996) *Annu Rev Neurosci* 19, 463-89.

(3) Stephens, R.M. et al. (1994) *Neuron* 12, 691-705.

(4) Marsh, H.N. et al. (2003) *J Cell Biol* 163, 999-1010.

(5) Obermeier, A. et al. (1993) *EMBO J* 12, 933-41.

(6) Obermeier, A. et al. (1994) *EMBO J* 13, 1585-90.

(7) Arevalo, J.C. et al. (2001) *Oncogene* 20, 1229-34.

(8) Reuther, G.W. et al. (2000) *Mol Cell Biol* 20, 8655-66.

(9) Greco, A. et al. (1997) *Genes Chromosomes Cancer* 19, 112-23.

(10) Pierotti, M.A. and Greco, A. (2006) *Cancer Lett* 232, 90-8.

(11) Lagadec, C. et al. (2009) *Oncogene* 28, 1960-70.

(12) Greco, A. et al. (2010) *Mol Cell Endocrinol* 321, 44-9.

(13) Ødegaard, E. et al. (2007) *Hum Pathol* 38, 140-6.

(14) Huang, E.J. and Reichardt, L.F. (2003) *Annu Rev Biochem* 72, 609-42.

(15) Geiger, T.R. and Peeper, D.S. (2005) *Cancer Res* 65, 7033-6.

(16) Han, L. et al. (2007) *Med Hypotheses* 68, 407-9.

(17) Aoyama, M. et al. (2001) *Cancer Lett* 164, 51-60.

(18) Desmet, C.J. and Peeper, D.S. (2006) *Cell Mol Life Sci* 63, 755-9.