#12651 Store at -20°C

PDGF Receptor Activation Antibody Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Akt (pan) (C67E7) Rabbit mAb	4691	20 µl	60 kDa	Rabbit IgG
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	4370	20 µl	44, 42 kDa	Rabbit IgG
p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb	4695	20 µl	42, 44 kDa	Rabbit IgG
Phospho-PDGF Receptor β (Tyr751) (C63G6) Rabbit mAb	4549	20 µl	190 kDa	Rabbit IgG
PDGF Receptor β (28E1) Rabbit mAb	3169	20 µl	190 kDa	Rabbit IgG
Phospho-SHP-2 (Tyr542) Antibody	3751	20 µl	72 kDa	Rabbit IgG
SHP-2 (D50F2) Rabbit mAb	3397	20 µl	72 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The PDGF Receptor Activation Antibody Sampler Kit provides an economical means to evaluate the activation status of multiple members of the PDGF receptor pathway, including SHP-2, Akt, and p44/42 MAPK (Erk1/2). The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Platelet derived growth factor (PDGF) family proteins form dimers (PDGF AA, PDGF AB, PDGF BB, PDGF CC, and PDGF DD) that bind receptor tyrosine kinases PDGF receptor α (PDGFRα) and PDGF receptor β (PDGFRβ) in a specific pattern. PDGFRβ homodimers bind PDGF BB and DD homodimers and the PDGF AB heterodimer. Heteromeric receptor PDGF α/β binds PDGF B, C, and D homodimers and the PDGF AB heterodimer (1). Ligand binding induces PDGF receptor dimerization and autophosphorylation, followed by binding and activation of cytoplasmic SH2 domain-containing signal transduction molecules, such as GRB2, Src, GAP, PI3 kinase, PLCγ, and NCK. Activated PDGF receptors initiate signaling pathways that control cell growth, actin reorganization, migration, and differentiation (2). PDGFRβ kinase-insert region residue Tyr751 forms the PI3 kinase docking site, and phosphorylation of PDGFRβ at this site inhibits the association between the SH2 domain of the PI3 kinase p85 subunit and PDGFRβ (3,4).

SHP-2 (PTPN11) is a nonreceptor protein tyrosine phosphatase that participates in signaling pathways that control cell growth, differentiation, migration, and death (5). Activation of SHP-2 and its association with Gab1 is critical for sustained Erk activation downstream of growth factor receptors and cytokines (6). Phosphorylation of SHP-2 at Tyr542 and Tyr580 in response to growth factor receptor activation is thought to relieve basal inhibition and stimulate SHP-2 tyrosine phosphatase activity (7,8).

Insulin and various growth/survival factors activate Akt, a kinase that acts in a wortmannin-sensitive pathway involving PI3 kinase to help control survival and apoptosis (9-11). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (12) and by phosphorylation within the carboxy terminus at Ser473.

The p44/42 MAPK (Erk1/2) signaling pathway is activated in response to extracellular stimuli including mitogens, growth factors, and cytokines (13-15). Research suggests that this pathway is an important target in cancer diagnosis and treatment (16). External stimuli lead to activation of a kinase cascade that results in the activation of p44 and p42 by a MAP kinase. MEK1 and MEK2 activate p44 and p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively.

Clinical studies describe PDGF expression in a number of different solid tumors, from glioblastomas to prostate carcinomas. The biological role of PDGF signaling in these tumors varies from autocrine stimulation of cancer cell growth to more subtle paracrine interactions involving adjacent stroma and even angiogenesis. Targeting PDGF signaling may be an effective way for tumor treatment (17).

Specificity/Sensitivity: Unless otherwise indicated, each antibody in the PDGF Receptor Activation Antibody Sampler Kit recognizes endogenous levels of its specific target. Activation state antibodies detect their intended targets only when phosphorylated at the indicated site. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb detects endogenous levels of p44 and p42 MAP kinase when dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185/Tyr187 of Erk2) and singly phosphorylated at Thr202. Phospho-PDGF Receptor β (Tyr751) (C63G6) Rabbit mAb may cross-react with activated PDGF receptor α and other protein tyrosine kinases when highly overexpressed. PDGF Receptor β (28E1) Rabbit mAb may cross-react with PDGF receptor α when highly overexpressed. Phospho-SHP-2 (Tyr542) Antibody may cross-react with activated receptor tyrosine kinases.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr542 of human SHP-2 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal activation state antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser473 of human Akt, Thr202/Tyr204 of human p44 MAP kinase, or Tyr751 of human PDGF receptor ß. Monoclonal control antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues in the carboxy-terminal sequence of mouse Akt, the carboxy terminus of rat p44 MAP kinase, the carboxy-terminus of human SHP-2, or a fusion containing a carboxy-terminal fragment of human PDGF receptor ß protein.

U.S. Patent No. 5,675,063

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**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Background References:

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.