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PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Antibody Pair

1 Kit (Reagents for 4 x 96 well plates)

Species Cross Reactivity: H M UniProt ID: #P09619 Entrez-Gene Id: #5159

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Volume	Cap Color	Storage Temp
PDGF Receptor β Capture Rabbit mAb (100X)	27128	400 μ l	Pink	+4C
Phospho-PDGF Receptor β (Tyr751) Detection Mouse mAb (100X)	32553	400 μ l	Blue	+4C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 μ l	Yellow	-20C

Please visit cellsignal.com for a complete listing of recommended companion products.

Description

CST's PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Kit #7345. Capture and detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The PDGF receptor β capture antibody is coated on a 96 well microplate in PBS overnight. After blocking, cell lysates are added followed by a phospho-PDGF receptor β (Tyr751) detection antibody and anti-mouse IgG, HRP conjugated antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-PDGF receptor β (Tyr751) protein.

Antibodies in kit are custom formulations specific to kit.

Reagents Not Supplied

Phosphate Buffered Saline (PBS-20X) #9808
Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
Cell Lysis Buffer (10X) #9803
TMB Substrate #7004
STOP Solution #7002
Blocking Buffer: 1X PBS/0.5% Tween-20, 1% BSA
96 Well Microplates**
Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background

Platelet derived growth factor (PDGF) family proteins exist as several disulphide-bonded, dimeric isoforms (PDGF AA, PDGF AB, PDGF BB, PDGF CC, and PDGF DD) that bind in a specific pattern to two closely related receptor tyrosine kinases, PDGF receptor α (PDGFR α) and PDGF receptor β (PDGFR β). PDGFR α and PDGFR β share 75% to 85% sequence homology between their two intracellular kinase domains, while the kinase insert and carboxy-terminal tail regions display a lower level (27% to 28%) of homology (1). PDGFR α homodimers bind all PDGF isoforms except those containing PDGF D. PDGFR β homodimers bind PDGF BB and DD isoforms, as well as the PDGF AB heterodimer. The heteromeric PDGF receptor α/β binds PDGF B, C, and D homodimers, as well as the PDGF AB heterodimer (2). PDGFR α and PDGFR β can each form heterodimers with EGFR, which is also activated by PDGF (3). Various cells differ in the total number of receptors present and in the receptor subunit composition, which may account for responsive differences among cell types to PDGF binding (4). Ligand binding induces receptor dimerization and autophosphorylation, followed by binding and activation of cytoplasmic SH2 domain-containing signal transduction molecules, such as GRB2, Src, GAP, PI3 kinase, PLC γ , and NCK. A number of different signaling pathways are initiated by activated PDGF receptors and lead to control of cell growth, actin reorganization, migration, and differentiation (5). Tyr751 in the kinase-insert region of PDGFR β is the docking site for PI3 kinase (6). Phosphorylated pentapeptides derived from Tyr751 of PDGFR β (pTyr751-Val-Pro-Met-Leu) inhibit the association of the carboxy-terminal SH2 domain of the p85 subunit of PI3 kinase with PDGFR β (7). Tyr740 is also required for PDGFR β -mediated PI3 kinase activation (8).

Background References

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5. Ostman, A. and Heldin, C.H. (2001) *Adv. Cancer Res.* 80, 1-38.
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 7. Ramalingam, K. et al. (1995) *Bioorg. Med. Chem.* 3, 1263-1272.
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#7826

PathScan[®] Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Antibody Pair

ELISA Antibody Pair

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 dH₂O, mix.
2. **Wash Buffer:** 1X PBS/0.05% Tween[®] 20, (20X PBST #9809).
3. **Blocking Buffer:** 1X PBS/0.05% Tween[®] 20, 1% BSA.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH₂O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA):** (#9998).
6. **TMB Substrate:** (#7004).
7. **STOP Solution:** (#7002)

NOTE: Reagents should be made fresh daily.

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 (x14,000 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 (~1,200 rpm) when the culture reaches 0.5-1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 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 (x14,000 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. Coating Procedure

1. Rinse microplate with 200 µl of dH₂O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hr).
3. **After overnight coating, gently uncover plate and wash wells:**
 1. Discard plate contents into a receptacle.
 2. Wash four times with wash buffer, 200 µl each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 μ l of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 μ l of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 μ l/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 μ l of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 μ l/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100 μ l of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 μ l of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
 1. **Visual Determination:** Read within 30 min after adding STOP solution.
 2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

posted January 2008

revised September 2013