

# PathScan® Phospho-PDGF Receptor $\alpha$ (Tyr849) Sandwich ELISA Antibody Pair

✓ 1 Kit  
(4 X 96 assays)



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New 03/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Entrez-Gene ID #5156  
Swiss-Prot Acc. # P16234

## Species Cross-Reactivity: H

**Description:** CST's PathScan® Phospho-PDGF Receptor  $\alpha$  (Tyr849) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-PDGF Receptor  $\alpha$  (Tyr849) Sandwich ELISA Kit #7296. 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 Phospho-PDGF  $\alpha$  Rabbit Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by PDGF  $\alpha$  Mouse Detection Antibody and HRP-conjugated Secondary 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  $\alpha$  (Tyr849) protein.

\*Antibodies in this kit are custom formulations specific to the kit.

## Reagents not supplied:

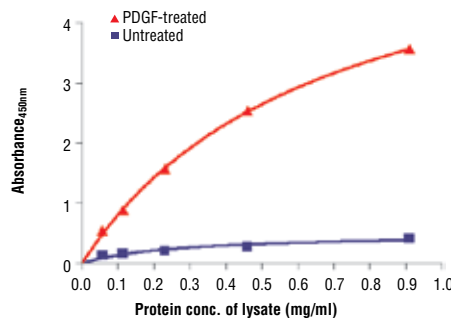
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
- Cell Lysis Buffer (10X) #9803
- TMB Substrate #7004
- STOP Solution #7002
- Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA
- 96 Well Microplates\*\*
- Microplate Reader

\*\* Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

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

**Background:** The proteins of the platelet derived growth factor (PDGF) family 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  $\alpha$  (PDGFR $\alpha$ ) and PDGF receptor  $\beta$  (PDGFR $\beta$ ). PDGFR $\alpha$  and PDGFR $\beta$  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). PDGF Receptor  $\alpha$  homodimers

| Products Included                                      | Volume  | Cap Color | Storage |
|--|---------|-----------|---------|
| PDGFR $\alpha$ (Tyr849) Rabbit Capture Antibody (100X) | 0.4 ml  | Pink      | 4°C     |
| PDGFR $\alpha$ Mouse Detection Antibody (100X)         | 0.4 ml  | Blue      | 4°C     |
| Anti-Mouse IgG HRP-Linked Antibody (1000X)             | 0.04 ml | Yellow    | -20°C   |



The relationship between protein concentration of untreated and PDGF-treated MG63 cell lysates and the absorbance at 450 nm is shown. Cells were serum starved overnight and then treated with PDGF (50 ng/ml) for 7 min. at 37°C.

bind all PDGF isoforms except those containing PDGF D. PDGF Receptor  $\beta$  homodimers bind PDGF BB and DD isoforms, as well as the PDGF AB heterodimer. The heteromeric PDGF  $\alpha/\beta$  receptor binds PDGF B, C, and D homodimers as well as the PDGF AB heterodimer (2). PDGFR $\alpha$  and PDGFR $\beta$  can each form heterodimers with EGFR, which is also activated by PDGF (3). Various cells differ in the total number of receptors present and 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 $\gamma$  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 $\beta$  is the docking site for PI3 kinase (6). Phosphorylated pentapeptides derived from Tyr751 of PDGFR $\beta$  (pTyr751-Val-Pro-Met-Leu) inhibit the association of the carboxy-terminal SH2 domain of the p85 subunit of PI3 kinase with PDGFR $\beta$  (7). Tyr740 is also required for PDGFR $\beta$  mediated PI3 kinase activation (8).

**Storage:** Capture and Detection Antibodies are stored at 4°C.

HRP-Conjugated Secondary Antibody is stored at -20°C.

## Companion Products:

- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809
- BSA #9998
- TMB Substrate #7004
- STOP Solution #7002
- Anti-mouse IgG, HRP-linked Antibody #7076
- PathScan® Phospho-PDGF Receptor  $\alpha/\beta$  (panTyr) Sandwich ELISA Kit #7235
- PathScan® Total PDGF Receptor  $\alpha$  Sandwich ELISA Kit #7318
- Cell Lysis Buffer (10X) #9803
- Phospho-PDGF Receptor  $\alpha$  (Tyr754) (23B2) Rabbit mAb #2992
- Phospho-PDGF Receptor  $\alpha$  (Tyr849)/PDGF Receptor  $\beta$  (Tyr857) (C43E9) Rabbit mAb #3170

## Background References:

- (1) Deuel, T.F. et al. (1988) *Biofactors* 1, 213–217.
- (2) Bergsten, E. et al. (2001) *Nat. Cell Biol.* 3, 512–516.
- (3) Betsholtz, C. et al. (2001) *Bioessays* 23, 494–507.
- (4) Coughlin, S.R. et al. (1988) *Prog. Clin. Biol. Res.* 266, 39–45.
- (5) Ostman, A. and Heldin, C.H. (2001) *Adv. Cancer Res.* 80, 1–38.
- (6) Panayotou, G. et al. (1992) *EMBO J.* 11, 4261–4272.
- (7) Ramalingam, K. et al. (1995) *Bioorg. Med. Chem.* 3, 1263–1272.
- (8) Kashishian, A. et al. (1992) *EMBO J.* 11, 1373–1382.

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIA®

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## PathScan® Sandwich ELISA Antibody Pair Protocol

### A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)  
3.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)  
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA),  
1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA),  
1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,  
1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

**NOTE:** Reagents should be made fresh daily

### B Coating Procedure

- Rinse microplate with dH<sub>2</sub>O. Add 200 μl of dH<sub>2</sub>O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
  - Discard plate contents into a receptacle.
  - Wash 4 times with Wash Buffer, 200 μl each time for each well. 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.
  - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

### C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm<sup>2</sup>) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 minutes 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.

### D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.