

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

✓ 1 Kit (96 assays)

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rev. 03/03/06

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

Species Cross-Reactivity: H, M

Introduction: CST's PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Phospho-PDGF Receptor β (Tyr751) protein. A PDGF Receptor β (7345-28D12) Rabbit mAb* has been coated onto the microwells. After incubation with cell lysates, Both nonphospho- and phospho- PDGF Receptor β proteins are captured by the coated antibody. Following extensive washing, Phospho-PDGF Receptor β Mouse mAb #3166* is added to detect the captured phospho-PDGF Receptor β protein. HRP-linked anti-mouse antibody #7076* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-PDGF Receptor β (Tyr751) protein.

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

Companion Products:

PDGF Receptor α Antibody #3164

PathScan® PDGFR Activity Assay: Phospho-PDGFR, Phospho-SHP2, Phospho-Akt, and Phospho-p44/42MAPK Multiplex Western Detection Kit #7180

PDGF Receptor β Antibody #3162

Phospho-PDGF Receptor β (Tyr751) (88H8) Mouse mAb #3166

Phospho-PDGF Receptor β (Tyr751) Antibody #3161

Platelet-Derived Growth Factor (PDGF) #9909

Specificity/Sensitivity: CST's PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA Kit detects endogenous levels of Phospho-PDGF Receptor β (Tyr751) protein. Using this Sandwich ELISA Kit #7345, a significant induction of phospho-PDGF Receptor β (Tyr751) in NIH/3T3 cells treated with PDGF can be detected. However, the level of total PDGF Receptor β protein remains unchanged, as shown by Western analysis using PDGF Receptor β Rabbit mAb (7345-28D12) [Fig.1].

Products Included	Volume	Solution Color
PDGF Receptor β (7345-28D12) Rabbit mAb Coated Microwells*	96 tests	
Phospho-PDGF Receptor β (Tyr751) Detection Ab	11 ml	green
Anti-mouse IgG HRP-Linked Ab	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

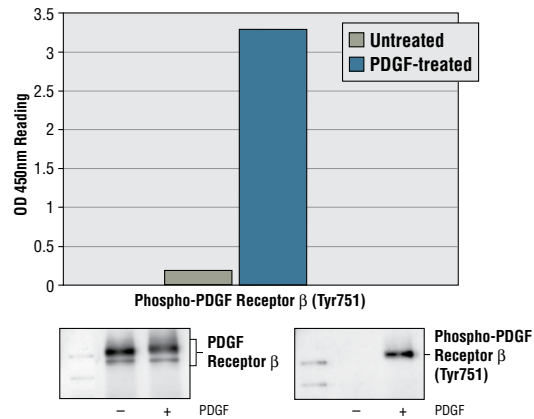


Figure 1: Treatment of NIH/3T3 cells with PDGF stimulates phosphorylation of PDGF Receptor β at Tyr751, detected by PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA kit, #7345, but does not affect the level of total PDGF Receptor β protein detected by PDGF Receptor β Rabbit mAb (7345-28D12) via Western analysis. OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-PDGF Receptor β (Tyr751) (88H8) Mouse mAb #3166 (right panel) or PDGF Receptor β Rabbit mAb (7345-28D12) (left panel), is shown in the bottom figure.



Background: The proteins of the PDGF family consist of several disulphide-bonded dimeric isoforms: PDGF AA, PDGF AB, PDGF BB, PDGF CC and PDGF DD, which bind in a distinct pattern to two highly related RTKs: PDGFR α and PDGFR β . PDGFR α and PDGFR β contain between 75% and 85% sequence homology between their two intracellular kinase domains. The kinase insert and carboxy-terminal tail regions display approximately 27% to 28% homology. PDGFR α binds all PDGF isoforms except PDGF D, whereas PDGFR β binds only PDGF B and D (1). PDGFR α and PDGFR β not only form homo- and heterodimers, but also dimerize with EGFR, which can be stimulated by PDGF (2). The total number and the ratio of receptor subunits expressed varies between cell types, possibly accounting for the difference in responsiveness of various cell types to PDGF (3). Ligand binding induces receptor dimerization and autophosphorylation, allowing binding and activation of cytoplasmic SH2 domain-containing signal transduction molecules including Grb2, Src, GAP, PI3 kinase, PLC γ and Nck. A number of different signaling pathways are thereby initiated leading to cell growth, actin reorganization, migration and differentiation (4). Tyr751 in the kinase-insert region of PDGFR β is the docking site for PI3 kinase (5). 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 (6). Tyr740 is also required for PDGFR β mediated PI-3 kinase activation (7).

Background References:

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

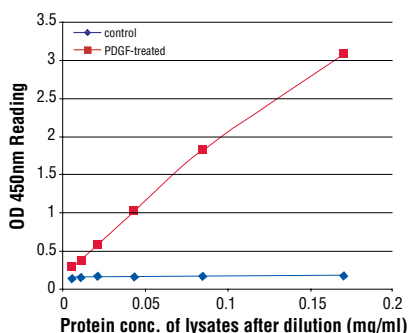


Figure 2: Linear relationship between protein concentration of lysates from control or PDGF-treated NIH/3T3 cells and kit assay optical density readings. NIH/3T3 cells (75% confluence) were treated with PDGF (50 ng/ml), and lysed after incubation at 37°C for 5 minutes.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X wash buffer (included in each Pathscan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #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 Na₂VO₄, 1 μg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm²) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. 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.

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. Add 100 μl of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.

3. Add 100 μl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X Wash Buffer, 200 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. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step C4.
7. Add 100 μl of HRP-Linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step C4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 μl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
 - a. Visual Determination — Read within 30 minutes after adding STOP Solution.
 - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.