

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

✓ 1 Kit (96 assays)



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

Species Cross-Reactivity: H, M

Description: CST's PathScan® Phospho-PDGF Receptor beta (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 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 is added to detect the captured phospho-PDGF Receptor β protein. Anti-mouse IgG, HRP-linked 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 beta (Tyr751) protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Phospho-PDGF Receptor beta (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]. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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 α (PDGFRα) and PDGF receptor β (PDGFRβ). PDGFRα and PDGFRβ 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). PDGFRα homodimers bind all PDGF isoforms except those containing PDGF D. PDGFRβ 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). PDGFRα and PDGFRβ 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

Product Includes	Item #	Kit Quantity	Color	Storage Temp
PDGFR beta Rabbit mAb Coated Microwells*	69122	96 tests		4°C
Phospho-PDGF Receptor Beta (Tyr751) Mouse Detection mAb	14002	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
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

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

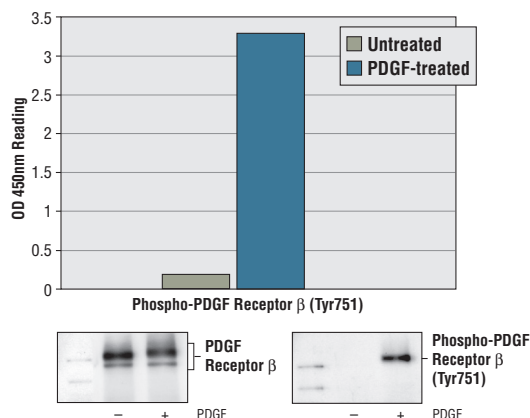


Figure 1: Treatment of NIH/3T3 cells with PDGF stimulates phosphorylation of PDGF Receptor β at Tyr751, detected by PathScan® Phospho-PDGF Receptor β (Tyr751) Sandwich ELISA kit, #7345, but does not affect the level of total PDGF Receptor β protein detected by PDGF Receptor β Rabbit mAb (7345-28D12) 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.

and activation of cytoplasmic SH2 domain-containing signal transduction molecules such as Grb2, Src, GAP, PI3 kinase, PLCγ, 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 PDGFRβ

is the docking site for PI3 kinase (6). Phosphorylated pentapeptides derived from Tyr751 of PDGFRβ (pTyr751-Val-Pro-Met-Leu) inhibit the association of the carboxy-terminal SH2 domain of the p85 subunit of PI3 kinase with PDGFRβ (7). Tyr740 is also required for PDGFRβ-mediated PI3 kinase activation (8).

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

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

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.

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.

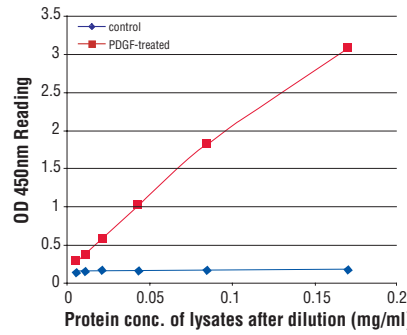


Figure 2: Linear relationship between protein concentration of lysates from control or PDGF-treated NIH/3T3 cells and kit assay optical density readings. NIH/3T3 cells (75% confluence) were treated with PDGF (50 ng/ml), and lysed after incubation at 37°C for 5 minutes.

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

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 1.0 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 1.0 ml volume of reconstituted Detection Antibody to 10.0 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 1.0 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 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 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 (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided 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 phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

**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 × 10⁶ 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 100 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 37°C. 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, 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.
 - d. Clean the underside of all wells with a lint-free tissue.
 5. Add 100 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
 6. Repeat wash procedure (Section C, Step 4).
 7. Add 100 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 for 30 min at 37°C.
 8. Repeat wash procedure (Section C, Step 4).
 9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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 min after adding STOP Solution.
 - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.