

PathScan® Phospho-PDGF Receptor α/β (panTyr) 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

Introduction: The PathScan® Phospho-PDGF Receptor α/β (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of PDGF receptor α/β when tyrosine phosphorylated. A PDGF Receptor α/β Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, PDGF Receptor α/β (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Biotinylated Phospho-Tyrosine Mouse Detection Antibody* is added to detect tyrosine phosphorylation of the captured PDGF receptor α/β protein. HRP-linked Streptavidin* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of PDGF receptor α/β phosphorylated on tyrosine.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Phospho-PDGF Receptor α/β (panTyr) Sandwich ELISA Kit #7235 detects PDGF receptor α/β when tyrosine phosphorylated. As shown in Figure 1, a significant induction of PDGF Receptor α/β tyrosine phosphorylation can be detected in MG63 cells following treatment with PDGF using the Phospho-PDGF Receptor α/β (panTyr) Sandwich ELISA Kit #7235. The level of total PDGF receptor α (phospho and nonphospho) remains unchanged as shown by Western analysis and by PathScan® Total PDGF Receptor α Sandwich ELISA Kit #7318. Western blot analysis of protein captured in the PDGF receptor α/β antibody coated microwell indicates that PDGF receptor α/β (phospho and nonphospho) has been captured (data not shown). The Western blot also shows a major band corresponding to the phospho-PDGF receptor α/β protein when biotinylated anti-tyrosine antibody is used as probe (see Figure 3).

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) which bind in a specific pattern to two highly related RTKs, PDGF receptor α (PDGFR α) and PDGF receptor β (PDGFR β). PDGFR α and PDGFR β share between 75% and 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 α homodimers bind all PDGF isoforms except those containing PDGF D. PDGF Receptor β homodimers bind PDGF BB and DD isoforms, as well as the PDGF AB heterodimer. The heteromeric PDGF α/β receptor binds PDGF B, C, and D homodimers as well as the PDGF AB heterodimer (2). PDGFR α and PDGFR β

Product Includes	Item #	Kit Quantity	Color	Storage Temp
PDGF Receptor α/β Rabbit mAb Coated Microwells*	57845	96 tests		4°C
Phospho-Tyrosine Mouse Detection mAb (Biotinylated)	14055	1 each	Green (Lyophilized)	4°C
HRP-linked Streptavidin (ELISA Formulated)	11805	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.

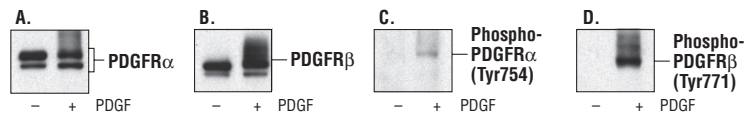
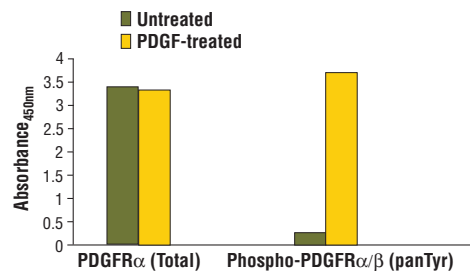


Figure 1. Treatment of MG63 cells with PDGF stimulates tyrosine phosphorylation of PDGFR α/β , detected by PathScan® Phospho-PDGF Receptor α/β (panTyr) Sandwich ELISA Kit #7235, but does not affect the level of total PDGFR α or β protein detected by either PathScan® Total PDGF Receptor α Sandwich ELISA Kit #7318 or Western analysis. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blot using PDGF Receptor α Rabbit mAb #3174 (panel A), PDGF Receptor β Rabbit mAb #3169 (panel B), Phospho-PDGF Receptor α (Tyr754) (23B2) Rabbit mAb #2992 (panel C) or Phospho-PDGF Receptor β (Tyr771) (76D6) Rabbit mAb #3173 (panel D) is shown in the bottom figure.

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 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:

- (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.

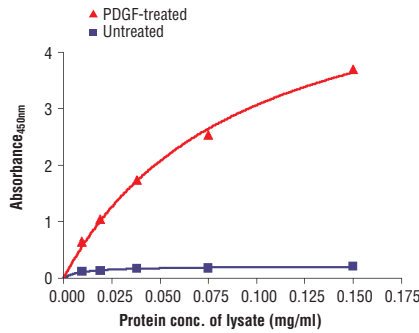


Figure 2. The relationship between protein concentration of lysates from untreated and PDGF-treated MG63 cells and the absorbance at 450 nm is shown. Cells (80% confluence) were serum starved overnight and then treated with PDGF (50 ng/ml) for 7 min. at 37°C.

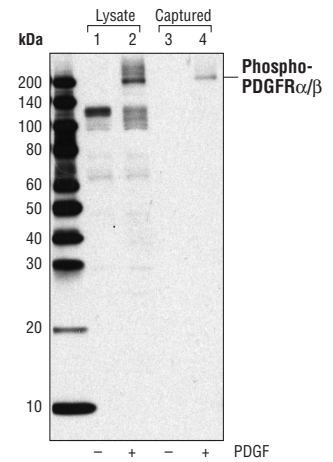


Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from MG63 cells and incubated in microwells coated with the PDGFR α/β capture antibody. Wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of MG63 cell starting lysate (lanes 1 & 2) and the captured protein (lanes 3 & 4) was performed using Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Biotinylated) #9417. The major band detected in the captured material corresponds to the Phospho-PDGFR α/β protein (lanes 3 & 4).

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- 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.
- 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.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 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 phenylmethylsulfonyl fluoride (PMSF) immediately before use.
- TMB Substrate** (#7004).
- 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.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- Remove media by low speed centrifugation (~1200 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.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- 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

- 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.
- 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.
- 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.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X 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.
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- 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.

- Read results.
 - Visual Determination:** Read within 30 min after adding STOP Solution.
 - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.