

# Phospho-PDGF Receptor $\alpha$ (Tyr849)/PDGF Receptor $\beta$ (Tyr857) (C43E9) Rabbit mAb

- Small 100  $\mu$ l  
(10 Western mini-blot)
- Petite 40  $\mu$ l  
(4 Western mini-blot)

New 10/07

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

**Orders** ■ 877-616-CELL (2355)  
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**Support** ■ 877-678-TECH (8324)  
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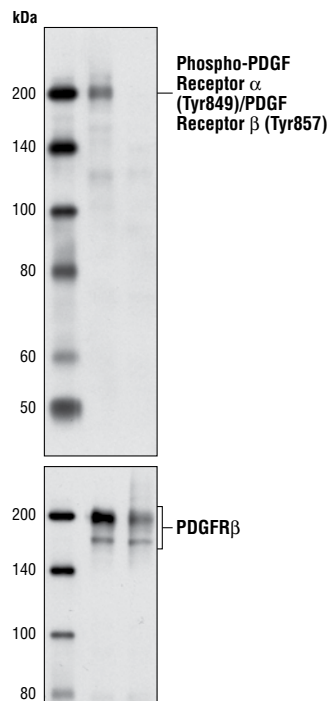
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Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W, IP	H, M, R	190 kDa	Rabbit**	IgG

**Background:** The proteins of the platelet derived growth factor (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: PDGF receptor  $\alpha$  (PDGFR $\alpha$ ) and PDGF receptor  $\beta$  (PDGFR $\beta$ ). PDGFR $\alpha$  and PDGFR $\beta$  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 $\beta$  binds all PDGF isoforms except PDGF D, whereas PDGFR $\alpha$  binds only PDGF B and D (1). PDGFR $\alpha$  and PDGFR $\beta$  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 $\gamma$  and Nck. A number of different signaling pathways are initiated leading to cell growth, actin reorganization, migration and differentiation (4). Tyr751 in the kinase-insert region of PDGFR $\beta$  is the docking site for PI3 kinase (5). 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 $\beta$  (6). Tyr740 is also required for PDGFR $\beta$  mediated PI3 kinase activation (7).

**Specificity/Sensitivity:** Phospho-PDGF Receptor  $\alpha$  (Tyr849)/PDGF Receptor  $\beta$  (Tyr857) (C43E9) Rabbit mAb detects endogenous levels of PDGF receptor  $\alpha$  and  $\beta$  only when phosphorylated on Tyr849 of PDGFR $\alpha$  and Tyr857 of PDGFR $\beta$ . This antibody may cross-react with other activated tyrosine kinases.

**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr857 of human PDGF receptor  $\beta$ .



Western blot analysis of NIH/3T3 cells untreated or treated with PDGF using Phospho-PDGF Receptor  $\alpha$  (Tyr849)/PDGF Receptor  $\beta$  (Tyr857) (C43E9) Rabbit mAb (upper) and PDGF Receptor  $\beta$  (28E1) Rabbit mAb #3169 (lower).

Entrez-Gene ID #5156, 5159  
Swiss-Prot Acc. #P16234, P09619

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by Western blot.

\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

#### Recommended Antibody Dilutions:

Western Blotting 1:1000  
Immunoprecipitation 1:50

#### Companion Products:

Platelet-Derived Growth Factor (PDGF) #9909  
Phospho-PDGF Receptor  $\beta$  (Tyr1009) (42F9) Rabbit mAb #3124  
PDGF Receptor  $\beta$  (28E1) Rabbit mAb #3169  
PDGF Receptor  $\beta$  (2B3) Mouse mAb #3175  
PDGF Receptor  $\beta$  Antibody #3162  
PDGF Receptor  $\alpha$  Antibody #3164  
Phospho-PDGF Receptor  $\alpha$  (Tyr754) (23B2) Rabbit mAb #2992  
Phospho-PDGF Receptor  $\beta$  (Tyr1021) (6F10) Rabbit mAb #2227  
Phospho-PDGF Receptor  $\beta$  (Tyr751) (88H8) Mouse mAb #3166  
Phospho-PDGF Receptor  $\beta$  (Tyr751) Antibody #3161  
Phospho-PDGF Receptor  $\beta$  (Tyr740) (32A9) Rabbit mAb #3168  
Phospho-PDGF Receptor  $\beta$  (Tyr771) (76D6) Rabbit mAb #3173  
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
Anti-rabbit IgG, HRP-linked Antibody #7074

#### 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.

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIAP®  
Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.

## Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g/ml}$  Leupeptin

**NOTE:** Add 1 mM PMSF immediately prior to use.

1. **Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
2. **Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
3. **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

### 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 to each plate (10 cm) and incubate the plates on ice for 5 minutes.
4. Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate samples on ice three times for 5 seconds each.
6. Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

### C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

1. Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
6. Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

### Cell Lysate Pre-Clearing (Optional)

1. Take 200  $\mu\text{l}$  cell lysate and add to either Protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry).
2. Incubate at 4°C for 30 – 60 minutes.
3. Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
4. Proceed to step 1 of Immunoprecipitation.