#7919 Store at -80°C

# HTScan® Mouse PDGF Receptor α Kinase Assay Kit

☑ 100 Assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

**Orders** ■ 877-616-CELL (2355)
orders@cellsignal.com
**Support** ■ 877-678-TECH (8324)
info@cellsignal.com
**Web** ■ www.cellsignal.com

rev. 07/17/07



| Products Included | Product # | Kit Quantity |
|---|---|---|
| Phospho-Tyrosine Mouse mAb (P-Tyr-100) | 9411 | 30 µl |
| HTScan® Tyrosine Kinase Buffer (4X) | 9805 | 15 ml |
| ATP (10 mM) | 9804 | 1 ml |
| DTT (1000x, 1.25M) | | 80 µl |
| FLT3 (Tyr589) Biotinylated Peptide | 1305 | 1.25 ml |
| PDGR Receptor α Kinase (mouse) | 7920 | 2 X 5 µg |

**Description:** The kit provides a means of performing kinase activity assays with recombinant mouse PDGFRα kinase. It includes active PDGFRα kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-tyrosine antibody for detection of the phosphorylated form of the substrate peptide.

**Peptide Core Sequence:** NEY*FY*V

**Molecular Weights:** Peptide substrate, Biotin-FLT3 (Tyr589): 1,945 Daltons. GST-mouse PDGFRα Kinase domain: 87 kDa.

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing mouse PDGFRα (Lys550-Leu1089) (GenBank Accession No. NM_011058) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** Biotinylated-FLT3 (Tyr589) Peptide was selected as PDGFRα kinase substrate from screening a panel of 150 pairs of tyrosine containing peptides as potential substrate candidates. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified mouse PDGFRα kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain. The specific activity of the PDGFRα kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify the mouse PDGFRα kinase activity using the PDGFRα substrate peptide provided in this kit. The PDGFRα kinase sensitivity to inhibitor staurosporine was measured using the PDGFRα substrate peptide provided in this kit [Fig.5].

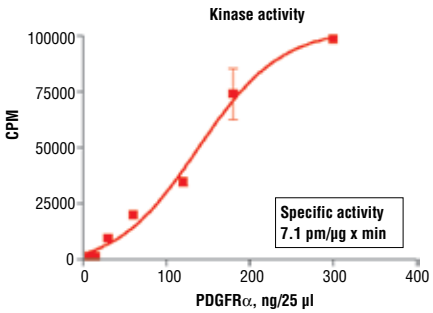


*Figure 1. Mouse PDGFRα kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 100 µM ATP, 250 µM FLT3 (Tyr589) biotinylated peptide and variable amount of Recombinant mouse PDGFRα. Reaction mixture incubated at room temperature for 10 minutes.*

**Storage:** Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

**Companion Products:**

PDGR Receptor α kinase (mouse) #7920

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

HTScan® Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

FLT3 (Tyr589) Biotinylated Peptide #1305

Tyrosine Kinase Substrate Screening Kit #7450

Staurosporine #9953

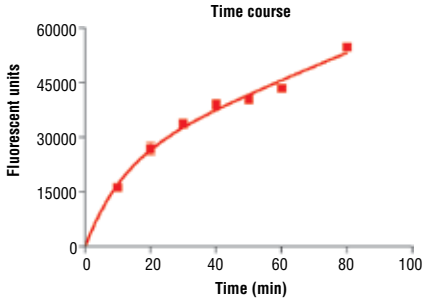


*Figure 2. Time course of mouse PDGFRα kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of PDGFRα substrate peptide (#1305) by the mouse PDGFRα kinase. In a 50 µl reaction, 60 ng PDGFRα and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

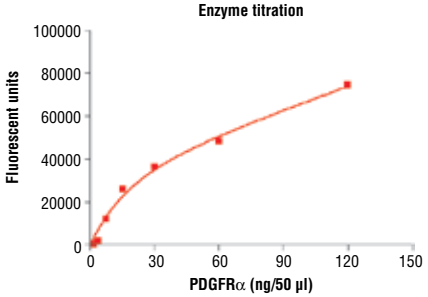


*Figure 3. Dose dependence curve of mouse PDGFRα kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1305) by mouse PDGFRα kinase. In a 50 µl reaction, increasing amounts of mouse PDGFRα and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

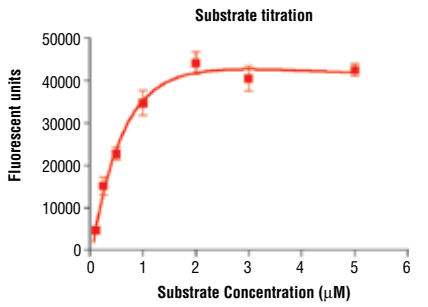


*Figure 4. Peptide concentration dependence of mouse PDGFRα kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1305) by the mouse PDGFRα kinase. In a 50 µl reaction, 60 ng of PDGFRα and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

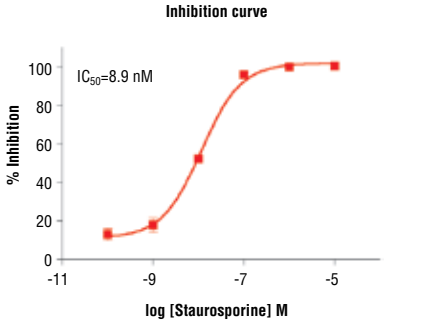


*Figure 5. Staurosporine inhibition of mouse PDGFRα kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of PDGFRα substrate peptide (#1305) by the mouse PDGFRα kinase. In a 50 µl reaction, 60 ng PDGFRα, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

**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: PDGFRα and PDGFRβ. PDGFRα and PDGFRβ 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. PDGFRα binds all PDGF isoforms except PDGF D, whereas PDGFRβ binds only PDGF B and D (1). PDGFRα and PDGFRβ 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, PLCγ 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 PDGFRβ is the docking site for PI3 kinase (5). 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 (6). Tyr740 is also required for PDGFRβ 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.

# Protocol for HTScan® Mouse PDGF Receptor α Kinase Assay Kit

## Kinase

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

## A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

*DELFIA® is a registered trademark of PerkinElmer Life Sciences*

## B Suggested Protocol for 100 Assays

1. Add 10 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with $dH_20$ to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=40 µM, [substrate]=3 µm).
2. Immediately transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan® Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM $Na_3VO_4$) to make DTT/Kinase buffer.
5. Transfer 0.6 ml of DTT/Kinase buffer to each enzyme tube to make 4X reaction cocktail ([enzyme] = 8 ng/µL in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

**Final Assay Conditions for a 50 µl Reaction**

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM $Na_3VO_4$
1.25 mM DTT
20 µM ATP
1.5 µM peptide
100 ng Mouse PDGF Recpetor α Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction and 75 µl $dH_2O$/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T
12. Dilute primary antibody, Phospho-Tyrosine mAb (P-Tyr-100), 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 60 minutes.
14. *Wash three times with 200 µl/well PBS/T
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

## DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

**Companion Products for DELFIA®**

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

## Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

**Companion Products For Colorimetric ELISA Assay**

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

*** NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com