

PDGFR β , Active

Recombinant protein expressed in Sf9 cells

Catalog # 7770-5

Lot# _____

Aliquot Size:	5 μ g in 50 μ l/vial
Concentration:	0.1 μ g/ μ l
Purity:	>90%
Storage:	-80°C
Shipping:	in Dry ice
Shelf Life:	6-12 months from shipping date
Specific Activity:	20 nmol/min/mg

Product Description

Recombinant human PDGFR β (557-end) was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM_002609](#).

Gene Aliases

JTK12; PDGFR; CD140B; PDGFR1; PDGF-R-beta

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

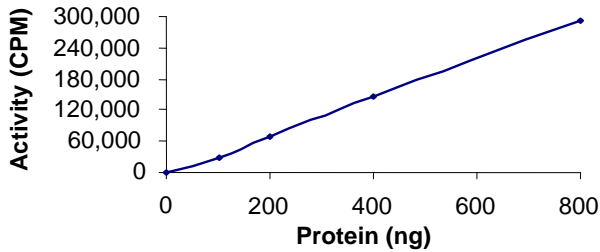
Scientific Background

PDGFR β (platelet-derived growth factor receptor β) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. PDGFR β deficient mice are hemorrhagic, severely anemic and exhibit a defect in kidney glomeruli function (1). However, absence of PDGFR β has no impact on major blood vessels and the heart. PDGFR β expression and activity is elevated in several cancers and inhibition of PDGFR β activity blocks progression of renal carcinoma in an animal model (2).

References

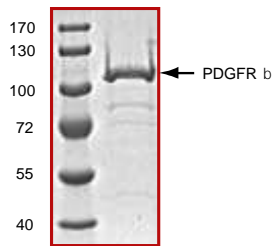
1. Soriano, P: Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev. 1994 Aug 15;8(16):1888-96.
2. Xu, L et al: Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression in an orthotopic mouse model. Cancer Res. 2005 Jul 1;65(13):5711-9.

Specific Activity



The specific activity of PDGFR β was determined to be **20 nmol / min / mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry.
Approx. MW **96kDa**.

Activity Assay Protocol

Reaction Components

Active Kinase #7770-5

Active PDGFR β (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PDGFR β for optimal results).

Kinase Dilution Buffer, pH 7.2

Kinase Assay Buffer II diluted at a 1:4 ratio (5X dilution) with 50 ng/ μ l BSA solution.

Kinase Assay Buffer II, pH 7.2

Buffer components: 25mM MOPS, 12.5mM β -glycerol-phosphate, 20mM MgCl₂, 25mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250 μ M [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution, 100 μ l [³²P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer. Store 1ml aliquots at -20°C.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200 μ l aliquots at -20°C.

Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1 mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PDGFR β , Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active PDGFR β .
 - Component 2.** 10 μ l of 1 mg/ml stock solution of substrate
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.

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- Step 5.** Initiate the reaction by the addition of 5 μ l [32 P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [32 P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of 32 P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]