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PDGFRβ, Active

Recombinant protein expressed in Sf9 cells

Catalog # 7770-5
Lot# _____

Aliquot Size:	5 µg in 50 µl/vial
Concentration:	0.1 µg/µl
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 PDGFRβ (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.

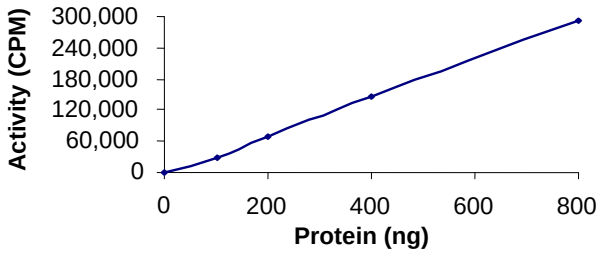
Scientific Background

PDGFRβ (platelet-derived growth factor receptor β) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. PDGFRβ deficient mice are hemorrhagic, severely anemic and exhibit a defect in kidney glome[illegible] function (1). However, absence of PDGFRβ has no impact on major blood vessels and the heart. PDGFRβ expression

References

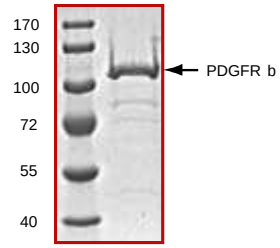
1. Soriano, P: Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev. 1994 Aug 15;8(16):1888-96.
2. Xu, L. et al: Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression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

Specific Activity



The specific activity of PDGFRβ 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 PDGFRβ (0.1μg/μl) 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 PDGFRβ for optimal results).

Kinase Dilution Buffer, pH 7.2

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

Kinase Assay Buffer II, pH 7.2

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 20mM $MgCl_2$, 25mM $MnCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution, 100μl [^{32P}]-ATP (1mCi/100μl), 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 200μl aliquots at –20°C.

Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1 mg/ml.

Assay Protocol

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Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active PDGFRβ.
- **Component 2.** 10μl 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 H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{32}P]-ATP Assay Cocktail bringing the final volume up to 25µl 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 20µl 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 H_2O) 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 5µl [^{32}P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

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

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