

PDGFR α , Active

Recombinant human protein expressed in Sf9 cells

Catalog # P12-18G

Lot # K1623-3

Product Description

Recombinant human PDGFR α (550-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_006206](#).

Gene Aliases

CD140A, PDGFR2, MGC74795, Rhe-PDGFR A

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

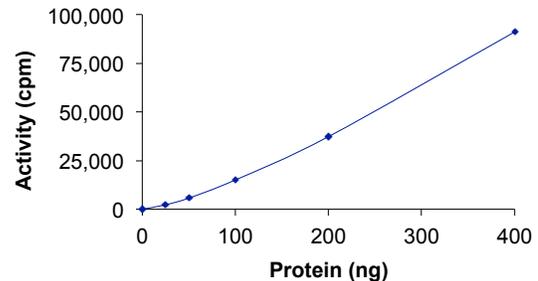
Scientific Background

PDGFR α (platelet-derived growth factor receptor α) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. Aberrant expression of PDGFR α has been linked to developmental abnormalities in vertebrate models and has been implicated in multiple disease states in humans. There is widespread expression of PDGFR α in renal cell types involved in fibrotic and sclerosing processes (1). PDGF and its receptor PDGFR α are inducers of fibrosis in the repair phase of inflammatory bowel disease and they may also be involved in the active inflammatory phase (2).

References

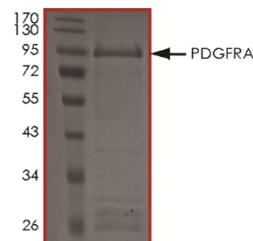
1. Floege, J. et al: Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. J Am Soc Nephrol. 1998 Feb;9(2):211-23.
2. Kumagai, S. et al: Platelet-derived growth factor and its receptors are expressed in areas of both active inflammation and active fibrosis in inflammatory bowel disease. Tohoku J Exp Med. 2001 Sep;195(1):21-33.

Specific Activity



The specific activity of PDGFR α was determined to be **12.5 nmol /min/mg** as per activity assay protocol.

Purity



The purity of human PDGFR α was determined to be **>90%** by densitometry. Approx. MW **95kDa**.

PDGFR α , Active

Recombinant human protein expressed in Sf9 cells

Catalog	P12-18G
Specific Activity	12.5 nmol/min/mg
Lot	K1623-3
Purity	>90%
Concentration	0.1µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P12-18G)

Active PDGFR α (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 α for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/ μ l BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 20mM MgCl₂, 25mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at -20°C

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: P61-58)

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

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PDGFR α , Kinase Assay Buffer, Substrate and Kinase 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 α (Catalog #P12-18G)
 - Component 2.** 5 μ l of 1 mg/ml stock solution of substrate (Catalog #P61-58)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- 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.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³³P]-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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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