

Product Information

PDGFR α (550-end), active, GST tagged, human Precisio® Kinase recombinant, expressed in Sf9 cells

Catalog Number **D0946**

Lot Number 070M0848

Storage Temperature -70°C

Synonyms: CD140A, MGC74795, Rhe-PDGFR α , platelet-derived growth factor receptor α

Product Description

PDGFR 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

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 006206. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~95 kDa

Purity: $\geq 70\%$ (SDS-PAGE, see Figure 1)

Specific Activity: 10–14 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 070M0848
 $>90\%$ (densitometry)

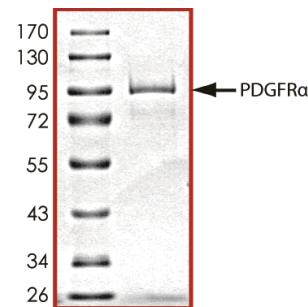
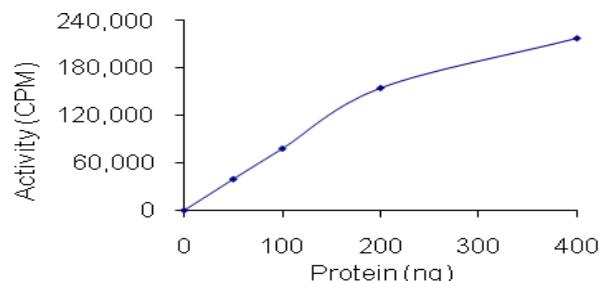


Figure 2.
Specific Activity of Lot Number 070M0848:
14 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 20 mM MgCl₂, 25 mM MnCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μl BSA solution.

Kinase Solution – Dilute the active PDGFR α (0.1 μ g/ μ l) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of active PDGFR α for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μ l aliquots at -20 °C.

γ -³²P-ATP Assay Cocktail (250 μ M) – Combine 5.75 ml of Kinase Assay Buffer, 150 μ l of 10 mM ATP Stock Solution, 100 μ l of γ -³²P-ATP (1 mCi/100 μ l). Store in 1 ml aliquots at -20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate Poly (Glu:Tyr, 4:1) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PDGFR α , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³²P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μ l:
10 μ l of Kinase Solution
5 μ l of Substrate Solution
5 μ l of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μ l of the γ -³²P-ATP Assay Cocktail, bringing the final reaction volume to 25 μ l. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ -³²P-ATP counts introduced into the reaction. Spot 5 μ l of the γ -³²P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 μ l of 250 μ M ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δ cpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Floege, J. et al., Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. *J. Am. Soc. Nephrol.*, **9**, 211-223 (1998).
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.*, **195**, 21-33 (2001).

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