

# PDGFR alpha (T674I), Active

Recombinant protein expressed in Sf9 cells

### Catalog # P12-12CG

Lot # \$261-1

### **Product Description**

Recombinant human PDGFR alpha (T674I) (550-end) was expressed by baculovirus in Sf9 insect cells using an Nterminal GST tag. The gene accession number is <u>NM 006206</u>.

### **Gene Aliases**

CD140A, PDGFR2, MGC74795, Rhe-PDGFRA

### 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**

PDGFRa (platelet-derived growth factor receptor a) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. Aberrant expression of PDGFRa has been linked to developmental abnormalities in vertebrate models and has been implicated in multiple disease states in humans. There is widespread expression of PDGFRa in renal cell types involved in fibrotic and sclerosing processes(1). PDGF and its receptor PDGFRa are inducers of fibrosis in the repair phase of inflammatory bowel disease and they may also be involved in the active inflammatory phase (2). PDGFRa(T674I) is one of the mutant forms of PDGFRa.

### 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.
- 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.

Catalogue #	Aliquot Size
P12-12CG -05	5 µg
P12-12CG -10	10 µg
P12-12CG -20	20 µg

### **Specific Activity**



The specific activity of PDGFR alpha (T674I) was determined to be **20 nmol /min/mg** as per activity assay protocol.

### **Purity**



The purity of PDGFR alpha (T674I) was determined to be >80% by densitometry, approx. MW 95 kDa.

## PDGFR alpha (T674I), Active

Recombinant protein expressed in Sf9 cells

Catalog Number Specific Activity Specific Lot Number

Purity Concentration Stability Storage & Shipping P12-12CG 20 nmol/min/mg S261-1 >80% 0.1 µg/µl 1yr At -70°C from date of shipment 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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: <u>orders@signalchem.com</u> <u>www.signalchem.com</u>

# Activity Assay Protocol

**Reaction Components** 

### Active Kinase (Catalog #: P12-12CG)

Active PDGFR alpha (T674I)  $(0.1 \mu g/\mu I)$  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 alpha (T674I) 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 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 20mM MgC1<sub>2</sub>, 25mM MnC1<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 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 $\mu$ l aliquots at -20°C.

Substrate (Catalog #: M42-51N)

MBP Protein substrate diluted in distilled  ${\rm H}_2{\rm O}$  to a final concentration of 1mg/ml.

### Assay Protocol

- Step 1. Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PDGFR alpha (T674I), 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 20μl:
  - Component 1. 10µl of diluted Active PDGFR alpha (T674I) (Catalog # P12-12CG)
  - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog # M42-51N)
  - Component 3. 5µl distilled H<sub>2</sub>O (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 H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5 μl [<sup>33</sup>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 20 μl 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<sub>2</sub>O) 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<sup>33</sup>]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: <u>orders@signalchem.com</u> www.signalchem.com

### FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.