

PDGFR α (T674I), Active

Recombinant human mutant protein expressed in Sf9 cells

Catalog # P12-12CG-10

Lot # S261-1

Product Description

Recombinant human PDGFR α (T674I) (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 α

Concentration

0.1 $\mu\text{g}/\mu\text{l}$

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, Shipping 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. Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

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). PDGFR α (T674I) is one of the mutant forms of PDGFR α .

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.

Purity

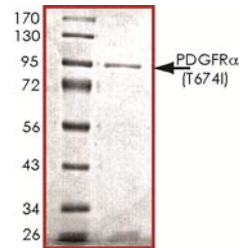
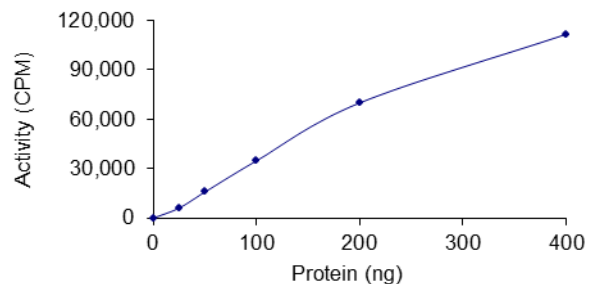


Figure 1. SDS-PAGE gel image

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

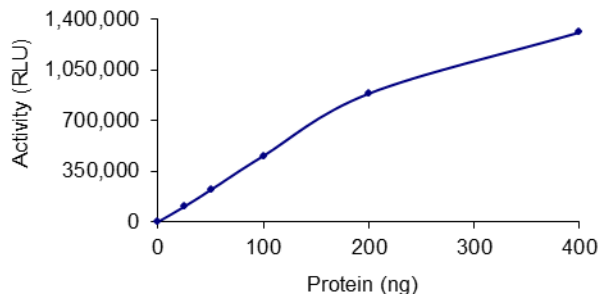
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of PDGFR α (T674I) was determined to be **20 nmol/min/mg** as per activity assay protocol. (For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of PDGFR α (T674I) was determined to be **70 nmol/min/mg** as per activity assay protocol. (For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P12-12CG)

Active PDGFR α (T674I) (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 PDGFRA (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/ 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 #: M42-51N)

MBP Protein 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 (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 20 μ l:
 - Component 1.** 10 μ l of diluted Active PDGFR α (T674I) (Catalog # P12-12CG)
 - Component 2.** 5 μ l of 1mg/ml stock solution of substrate (Catalog # M42-51N)
 - 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., 1,250 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)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

PDGFR α (T674I) Kinase Enzyme System (Promega, Catalog #:V4486)

PDGFR α (T674I), Active, 10 μ g (0.1 μ g/ μ l)
MBP Protein, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT solution (0.1M), 25 μ l
MnCl₂ solution (2.5M), 25 μ l

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl₂ and 0.5 μ g/ μ l BSA.

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)
ADP, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Assay Protocol

The PDGFR α (T674I) assay is performed using the PDGFR α (T674I) Kinase Enzyme System (Promega; Catalog #: V4486) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The PDGFR α (T674I) reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the *ADP-Glo™ Kinase Assay*, see the Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of PDGFR α (T674I) Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400 μ l Reaction Buffer A, 1 μ l DTT, 1.6 μ l MnCl₂ and 597.4 μ l of dH₂O.
- Step 4.** Prepare 1ml of 250 μ M ATP Assay Solution by adding 25 μ l ATP solution (10mM) to 500 μ l of 2X Buffer and 475 μ l of dH₂O.
- Step 5.** Prepare diluted PDGFR α (T674I) in 1X Buffer (diluted from 2X buffer) 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 (T674I) for optimal results).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), 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 (T674I)
Component 2.	5 μ l of 1mg/ml stock solution of substrate
Component 3.	5 μ l of 2X Buffer
- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 8.** At the same time as the PDGFR α (T674I) kinase reaction, set up an ATP to ADP conversion curve at 50 μ M ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* Technical Manual #TM313.
- Step 9.** Initiate the PDGFR α (T674I) reactions by the addition of 5 μ l of 250 μ M ATP Assay Solution thereby bringing the final volume up to 25 μ l. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25 μ l of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50 μ l of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)