

p38gamma, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # **M37-10BG-10**

Lot # P118-1

Product Description

Recombinant full-length human p38 γ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_002969](#).

Gene Aliases

ERK3; ERK6; SAPK3; PRKM12; MAPK12

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

p38 γ is a member of the p38 MAPK family which is activated in response to stress (1). p38 γ gene was mapped to 22q13.3 and functions as a signal transducer during differentiation of myoblasts to myotubes. Enforced localization of p38 γ in the nucleus or cytoplasm markedly attenuates the ability of the kinase to induce cell cycle arrest in fibroblasts. p38 γ increases basal glucose uptake and decreases DNP- and contraction-stimulated glucose uptake, partially by affecting levels of glucose transporter expression in skeletal muscle (2).

References

- Li, Z. et al: The primary structure of p38-gamma: a new member of p38 group of MAP kinases. *Biochem. Biophys. Res. Commun.* 228: 334-340, 1996.
- Ho, RC. et al: p38gamma MAPK regulation of glucose transporter expression and glucose uptake in L6 myotubes and mouse skeletal muscle. *Am J Physiol Regul Integr Comp Physiol.* 2004 Feb; 286(2):R342-9. Epub 2003 Oct 30.

Purity

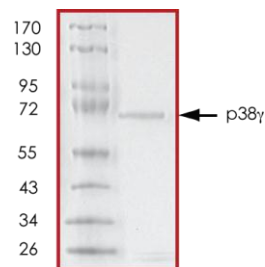
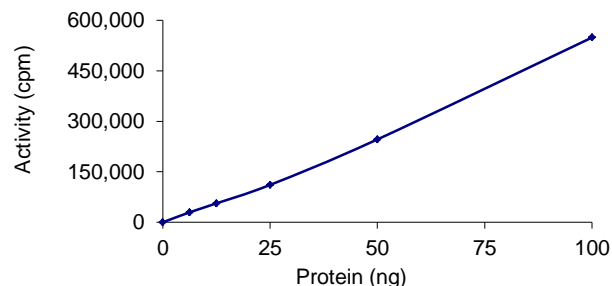


Figure 1. SDS-PAGE gel image

The purity of p38 γ was determined to be **>90%** by densitometry. Approx. MW **~71kDa**.

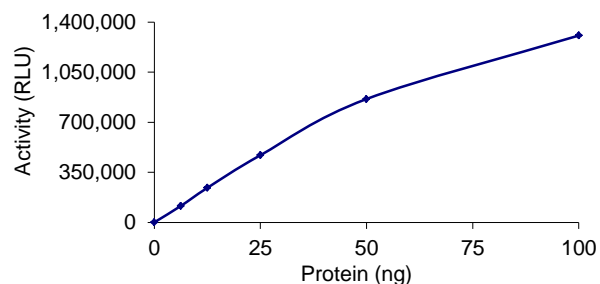
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of p38 γ was determined to be **220 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 p38 γ was determined to be **240 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 #: M37-10BG-10)

Active p38 γ (0.1 $\mu\text{g}/\mu\text{l}$) diluted with Kinase Dilution Buffer I (Catalog #: K21-09) 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 p38 γ for optimal results).

Kinase Dilution Buffer I (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H₂O.

Kinase Assay Buffer I (Catalog #: K01-09)

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

[³³P]-ATP Assay Cocktail

Prepare 250 μM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μl [³³P]-ATP (1mCi/100 μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution, pH7.2 (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 μl aliquots at -20°C.

Substrate (Catalog #: P03-58)

P38 Sub synthetic peptide (IPTTPITTYFFFKKK) diluted in distilled H₂O to a final concentration of 1mg/ml.

Note: MBP protein (Catalog #M42-51N) has also been previously used as a substrate for this target and it showed good activity.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active p38 γ , 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 μl :
 - Component 1.** 10 μl of diluted Active p38 γ (Catalog #M37-10BG)
 - Component 2.** 5 μl of 1mg/ml stock solution of substrate (Catalog #P03-58)
 - Component 3.** 5 μl of 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 μl [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μl 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 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 (cpm) 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 μl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

p38 γ Kinase Enzyme System (Promega, Catalog #:V3371)

p38 γ , Active, 10 μ g (0.1 μ g/ μ l)
p38 Substrate, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT solution (0.1M), 25 μ l

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)

Reaction Buffer A (5X)

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

Assay Protocol

The p38 γ assay is performed using the p38 γ Kinase Enzyme System (Promega; Catalog #: V3371) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The p38 γ 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 p38 γ 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 and 599 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 p38 γ 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 p38 γ 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 p38 γ
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 p38 γ 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 p38 γ 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 RLU, 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)