Recombinant Human Active p38α Protein, CF

Catalog Number: 5477-KS-010
Lot Number: 1779666

SPECIFICATIONS AND USE

Source	Recombinant full-length human p38 alpha (p38α) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_139012.
Molecular Mass	The approximate molecular weight is 67 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of p38α was determined to be 35 nmol/min/mg as per Activity Assay Protocol.
Storage	This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

p38α (SAPK2A) is a member of the p38 MAPK family which are activated by various environmental stresses and pro-inflammatory cytokines (1). The activation of p38 requires its phosphorylation by MAP kinase kinases (MKKs) or its autophosphorylation triggered by the interaction of MAP3K7IP1/TAB1 protein with this kinase (2). The substrates of p38 include transcription regulator ATF2, MEF2C, MAX, cell cycle regulator CDC25B, and tumor suppressor p53, which suggest the roles of this kinase in stress related transcription and cell cycle regulation, as well as in genotoxic stress response.

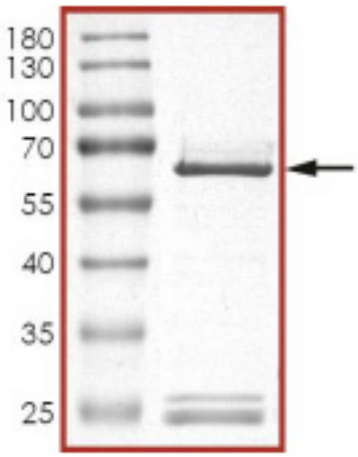

Figure 1: The approximate molecular weight is 67 kDa and the purity is > 90%.

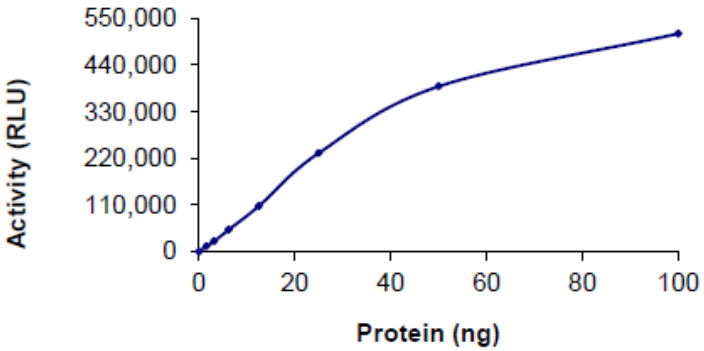

Figure 2: The specific activity of p38α was determined to be 35 nmol/min/mg as per Activity Assay Protocol.

REFERENCES

1. Han, J. *et al*. (1994) Science **265**:808.
2. Ge, B. *et al*. (2002) Science **295**:1291.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active p38α (0.1 µg/µL) diluted with Kinase Dilution Buffer IX as shown in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - p38 Sub synthetic (IPTTPITTTYFFFKKK) diluted in distilled 20 mM Tris HCl, pH 7.5 solution to a final concentration of 1 mg/mL.
Note: *ATF2 protein has also been previously used as a substrate for this target and it showed good activity.*

ASSAY PROCEDURE

The p38α assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the p38α reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active p38α, Kinase Assay Buffer lll (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1X), in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 µM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	79 µL
Substrate at 1 mg/mL	80 µL

3. Transfer the following reaction components prepared in step 1 and step 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active p38α
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]