Recombinant Human Active p38α

Certificate of Analysis

Catalog Number: 5477-KS
Lot Number: 1497404

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 (see Figure 1 below).
Purity	♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
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 190 nmol/min/mg using a synthetic peptide substrate (see 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.**

p38α

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.

References

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

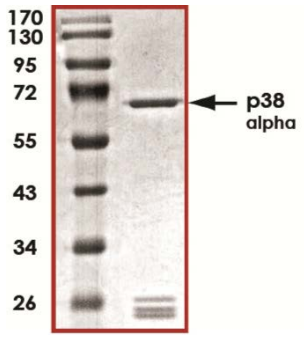

Figure 1: The approximate molecular weight is 67 kDa and the purity is > 95%.

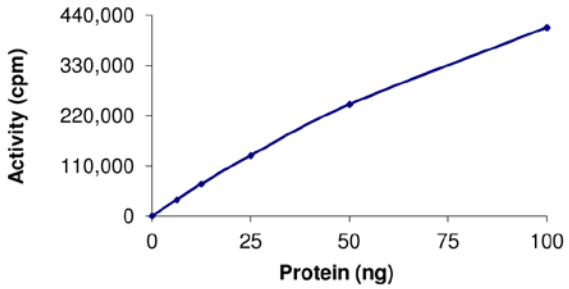

Figure 2: Enzymatic assay results. The specific activity of p38α was determined to be 190 nmol/min/mg as per the activity assay protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active p38α (0.1 µg/µL) diluted with Kinase Dilution Buffer III 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 I, pH 7.2** - 25 mM MOPS (pH 7.2), 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.
- **Kinase Dilution Buffer III** - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50 ng/µL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20 °C.
- **[^{33}P]-ATP Assay Cocktail** - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [^{33}P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I. Store in 1.0 mL aliquots at ≤ -20 °C.
- **Substrate** – P38 Sub synthetic peptide (IPTTPITTTYFFFKKK) diluted in distilled H_2O to a final concentration of 1.0 mg/mL
  Note: ATF2 protein has also been previously used as a substrate for this target and it showed good activity.

Assay Procedure

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active p38α, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer III on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active p38α	10 µL
Substrate (1.0 mg/mL Stock Solution)	5.0 µL
Distilled Water (4 °C)	5.0 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5 µL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
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.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity (cpm) 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 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)
Specific Activity (SA) = cpm for 5.0 µL [^{33}P]-ATP/pmole of ATP (in 5.0 µL of a 250 µM ATP stock solution, *i.e*. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minute/µg or nmol/minute/mg)
Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

Diane Wotta
Diane R. Wotta
Quality & Regulatory Affairs