**p38α, Active**
Human recombinant protein expressed in Sf9 cells

**Catalog # M39-10BG**
Lot # R122-2

**Product Description**

Recombinant full-length human p38α was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is **NM_139012**.

**Gene Aliases**

CSBP1; CSBP2; CSPB1; PRKM14; PRKM15; SAPK2A; MAPK14

**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**

p38α (SAPK2A) is a member of the p38 MAPK family which are activated by various environmental stresses and proinflammatory 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 (5).

**References**


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**Specific Activity**

The specific activity of p38α was determined to be **200 nmol/min/mg** as per activity assay protocol.

**Purity**

The purity of p38α was determined to be **>90%** by densitometry. Approx. MW **67kDa**.

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**Activity Assay Protocol**

**Reaction Components**

**Active Kinase** (Catalog #: M39-10BG)
Active p38α (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 p38α for optimal results).

**Kinase Dilution Buffer III** (Catalog #: K23-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

**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 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). 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 I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

**Substrate** (Catalog #: P03-58)
P38 Sub synthetic peptide (IPITPITTTYFFKKK) diluted in distilled H₂O to a final concentration of 1mg/ml.

**Note:** ATF2 protein (Catalog #: A10-55G) 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 p38α, 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 p38α (Catalog #: M39-10BG)
- Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #: P03-58)
- Component 3. 5µl 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 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 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 [P³³]-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 pmol)

**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) ]

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