

Product Information

p38 α , active, GST tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number **A4861**

Storage Temperature -70°C

Synonyms: CSBP1, CSBP2, CSPB1, PRKM14, PRKM15, SAPK2A, MAPK14

Product Description

p38 α is a member of the p38 MAPK family which is activated by various environmental stressors and proinflammatory cytokines.¹ The activation of p38 requires phosphorylation by MAP kinase kinases (MKKs) or 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.²

This recombinant product was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST-tag. The gene accession number is NM 139012. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: \sim 67 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
 \geq 70% (SDS-PAGE, densitometry)

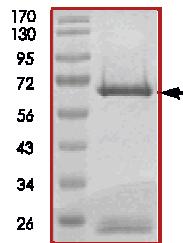
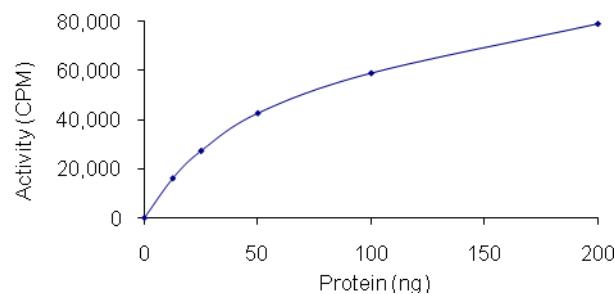


Figure 2.
Specific Activity of Typical Lot:
148–202 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μ l BSA solution.

Kinase Solution – Dilute the active p38 α (0.1 μ g/ μ l) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active p38 α for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μ l aliquots at -20 °C.

γ -³²P-ATP Assay Cocktail (250 μ M) – Combine 5.75 ml of Kinase Assay Buffer, 150 μ l of 10 mM ATP Stock Solution, 100 μ l of γ -³²P-ATP (1 mCi/100 μ l). Store in 1 ml aliquots at -20 °C.

Substrate Solution – Prepare ATF2 substrate in buffer (50 mM Tris-HCl, pH 7.2, 50 mM NaCl, 5 mM EDTA and 0.25 mM DTT) to a final concentration of 0.5 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active p38 α , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³²P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μ l:
10 μ l of Kinase Solution
10 μ l of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 μ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μ l of the γ -³²P-ATP Assay Cocktail, bringing the final reaction volume to 25 μ l. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ -³²P-ATP counts introduced into the reaction. Spot 5 μ l of the γ -³²P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity 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 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 μ l of 250 μ M ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δcpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Han, J. et al., A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science*, **265**, 808-811 (1994).
2. Ge, B. et al., MAPKK-independent activation of p38-alpha mediated by TAB1-dependent autophosphorylation of p38-alpha. *Science*, **295**, 1291-1294 (2002).

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