

Product Specification

p38 δ , active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7754-5
 Lot #: _____
 Aliquot size: 5 μ g protein in 50 μ l
 Specific activity: 259 nmol/min/mg

Quality Control Analysis

Activity assessment

p38delta protein (~100 ng/ μ l concentration) was diluted to 25ng/ μ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT and 40ng/ μ l BSA), followed by 2-fold serial dilutions, and then the 10 μ l diluted proteins were used to phosphorylate the MBP protein in the following assay condition:

- 10 μ l diluted p38delta protein
- 10 μ l MBP protein (2 mg/ml stock)
- 5 μ l [³²P] ATP mixture (250 μ M ATP, 0.16 μ Ci/ μ l in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30^oC and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 μ l of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

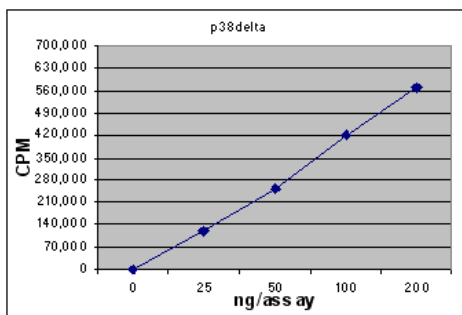


Fig. 1 p38delta activity assay

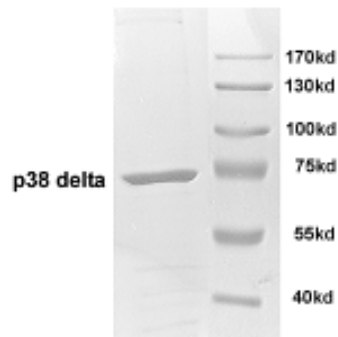


Fig. 2 p38delta protein gel

Purity assessment

1.5 μ g of p38delta protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the blue gel showed >80% purity of the p38delta protein product, and the band was at ~71kDa (Fig. 2).

Product Description

Recombinant full length human p38delta containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_002754.

This material is sold for research purposes only.

Specific Activity

259 nmol phosphate incorporated into MBP protein per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay).

Formulation

Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

Mitogen-activated protein kinase (MAPK) cascades represent one of the major signal systems used by eukaryotic cells to transduce extracellular signals into cellular responses, and involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. The stress-activated protein kinase 4 (SAPK4), or p38 delta, is a member of the MAPK family that are activated by chemical and environmental stresses as well as by proinflammatory cytokines. SAPK4 has a TGY dual phosphorylation motif and is activated in response to cellular stresses and proinflammatory cytokines (1). MAP kinase kinases 3, and 6 can phosphorylate and activate this kinase. Transcription factor ATF2, and microtubule dynamics regulator stathmin have been shown to be the substrates of this kinase (2).

1. Goedert, M.; Cuenda, A.; Craxton, M.; Jakes, R.; Cohen, P. Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. *EMBO J.* 16: 3563-3571, 1997.

2. Kumar S, McDonnell PC, Gum RJ, Hand AT, Lee JC, Young PR. Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. *Biochem Biophys Res Commun.* 1997 Jun 27;235(3):533-8.