

Product Specification

MAPKAP kinase 2, active

(Recombinant human protein, residues 46-end, expressed in Sf 9 cells)

Catalog #: 7737-5
Lot #: B150-1
Aliquot size: 5 µg protein in 50 µl
Specific activity: 254 nmol/min/mg

Quality Control Analysis

Activity assessment

MAPKAP kinase 2 protein (100 ng/µl concentration) was diluted to 20 ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 30 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the MAPKAP kinase 2 substrate peptide (KKLNRTLVA) in the following assay condition:

10 µl diluted MAPKAP kinase 2 protein
 10 µl MAPKAP kinase 2 substrate peptide (1 mg/ml stock)
 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30° C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl 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.

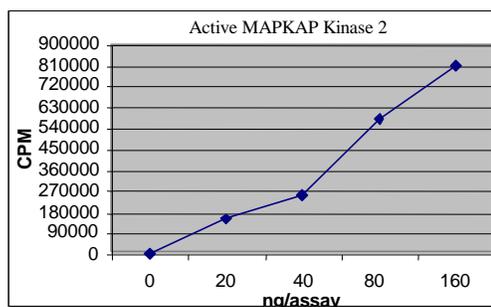


Fig. 1 MAPKAPK2 activity assay

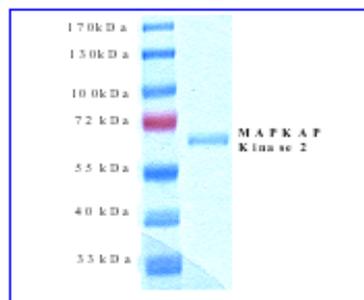


Fig. 2 MAPKAPK2 protein gel

Purity assessment

1 µg of MAPKAP kinase 2 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the MAPKAP kinase 2 product, and the band was at ~68 kDa (Fig. 2).

Product Description

Recombinant human MAPKAP kinase 2 protein (46-end) containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_032960.

This material is sold for research purposes only.

Specific Activity

254 nmol phosphate incorporated into MAPKAP kinase 2 substrate peptide per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP and total of 0.83 µCi/µl P-32.

Formulation

Recombinant protein 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° . Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

MAPKAP kinase2 is a Ser/Thr protein kinase, which is regulated through direct phosphorylation by p38 MAP kinase. In conjunction with p38 MAP kinase, this kinase is known to be involved in many cellular processes including stress and inflammatory responses, nuclear export, gene expression regulation and cell proliferation. Heat shock protein HSP27 was shown to be one of the substrates of this kinase in vivo.

Reference

1. Stokoe, D.; Caudwell, B.; Cohen, P. T. W.; Cohen, P.: The substrate specificity and structure of mitogen-activated protein (MAP) kinase-activated protein kinase-2. *Biochem. J.* 296: 843-849, 1993.
2. Maizels, E. T.; Mukherjee, A.; Sithanandam, G.; Peters, C. A.; Cottom, J.; Mayo, K. E.; Hunzicker-Dunn, M.: Developmental regulation of mitogen-activated protein kinase-activated kinases-2 and -3 (MAPKAPK-2/-3) in vivo during corpus luteum formation in the rat. *Molec. Endocr.* 15: 716-733, 2001.
3. McCormick, C.; Ganem, D.: The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. *Science* 307: 739-741, 2005.