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Product Specification

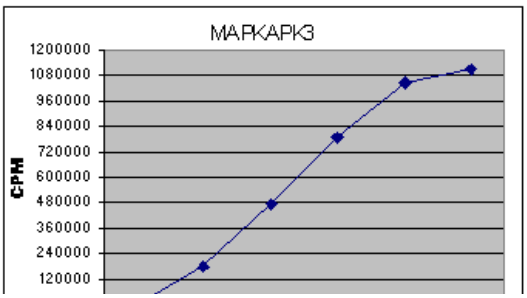
MAPKAPK3, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7755-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 636 nmol/min/mg

Quality Control Analysis
Activity assessment
MAPKAPK3 protein (~100 ng/µl concentration) was diluted to 22.5ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the MBP protein in the following assay condition:

10 µl diluted MAPKAPK3 protein
10 µl MBP protein (2 mg/ml stock)
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] 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.



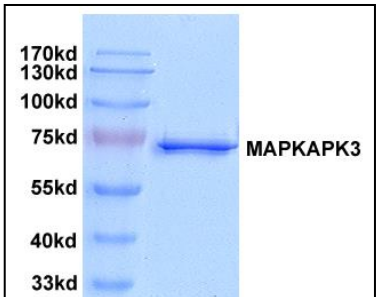


Fig. 1 MAPKAPK3 activity assay

Fig. 2 MAPKAPK3 protein gel

Purity assessment
1.5 µg of MAPKAPK3 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the blue gel showed >90% purity of the MAPKAPK3 protein product, and the band was at ~69 kDa (Fig. 2).

Product Description
Recombinant full length human MAPKAPK3 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_004635.
This material is sold for research purposes only.

Specific Activity
636 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
MAPKAPK3 has a single potential SH3-binding site in the proline-rich N terminus, a putative ATP-binding site, 2 MAP kinase phosphorylation site motifs, and a putative nuclear localization signal. It shares 72% nucleotide and 75% amino acid identity with MAPKAPK2 (1). MAPKAPK3 was shown to be activated by growth inducers and stress stimulation of cells. In vitro studies demonstrated that ERK, p38 MAP kinase and Jun N-terminal kinase were all able to phosphorylate and activate this kinase, which suggested the role of this kinase as an integrative element of signaling in both mitogen and stress responses (2). This kinase was reported to interact with, phosphorylate and repress the activity of E47, which is a basic helix-loop-helix transcription factor known to be involved in the regulation of tissue-specific gene expression and cell differentiation (3). MAPKAPK3 is uniquely poised to support luteal maturation through the phosphorylation and activation of the nuclear transcription factor CREB (4).

Reference:
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2. Ludwig, S.; Engel, K.; Hoffmeyer, A.; Sithanandam, G.; Neufeld, B.; Palm, D.; Gaestel, M.; Rapp, U.: 3pK, a novel mitogen-activated protein (MAP) kinase-activated protein kinase, is targeted by three MAP kinase pathways. *Molec. Cell. Biol.* 16: 6687-6697, 1996.
3. Neufeld B, Grosse-Wilde A, Hoffmeyer A, Jordan BW, Chen P, Dinev D, Ludwig S, Rapp UR. Serine/Threonine kinases 3pK and MAPK-activated protein kinase 2 interact with the basic helix-loop-helix transcription factor E47 and repress its transcriptional activity.J Biol Chem. 2000 Jul 7; 275(27):20239-42.
4. 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