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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
M42-10G -05	5 µg
M42-10G -10	10 µg
M42-10G -20	20 µg

MAPKAPK5, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # M42-10G
Lot # M036-2

Product Description

Recombinant full-length human MAPKAPK5 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_003668.

Gene Aliases

PRAK

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.

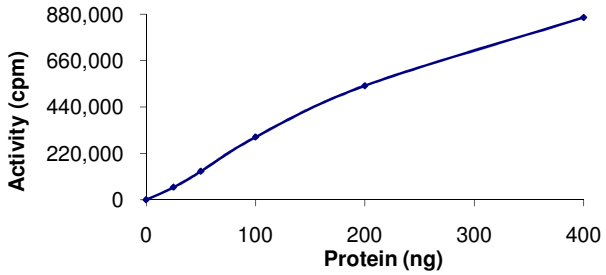
Scientific Background

MAPKAPK5 is a member of the serine/threonine kinase family that responds to cellular stress and proinflammatory cytokines. MAPKAPK5 is activated through its phosphorylation by MAP kinases including MAPK1/ERK, MAPK14/p38-alpha, and MAPK11/p38-beta (1). MAPKAPK5 is activated in HeLa cells in response to cellular stress and proinflammatory cytokines. MAPKAPK5 activity is regulated by p38-alpha and p38-beta both *in vitro* and *in vivo*, and thr-182 is the regulatory phosphorylation site of MAPKAPK5 (2). *In vitro*, MAPKAPK5 kinase phosphorylates heat shock protein HSP27 at its physiologically relevant sites.

References

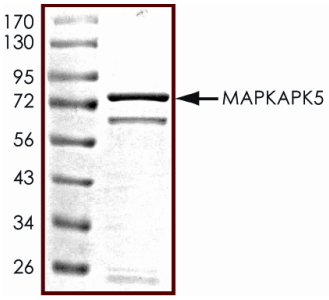
1. New, L.; Jiang, Y. et al: PRAK, a novel protein kinase regulated by the p38 MAP kinase. EMBO J. 17: 3372-3384, 1998.
2. Ni, H.; Wang. et al: MAPKAPK5, a novel mitogen-activated protein kinase (MAPK)-activated protein kinase, is a substrate of the extracellular-regulated kinase (ERK) and p38 kinase. Biochem. Biophys. Res. Commun. 243: 492-496, 1998.

Specific Activity



The specific activity of MAPKAPK5 was determined to be **172 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MAPKAPK5 was determined to be **>80%** by densitometry, approx. MW **79kDa**.

MAPKAPK5, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	M42-10G
Specific Activity	172 nmol/min/mg
Specific Lot Number	M036-2
Purity	>80%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M42-10G)

Active MAPKAPK5 (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 MAPKAPK5 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 final 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-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 #: H31-58)

HSP27tide synthetic peptide substrate (RRLNRQLSVA-amide) diluted to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active MAPKAPK5, 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 MAPKAPK5 (Catalog # M42-10G)
- **Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog # H31-58)
- **Component 3.** 5µl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{33}P]-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 20µl 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 H_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [^{33}P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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