MAPKAPK5, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # M42-10G-10
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

Concentration

0.1 µg/µl

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage, Shipping 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. Stability is 1yr at –70°C from date of shipment. Product shipped on dry ice.

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


Purity

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

Specific Activity

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

For Radiometric Assay Protocol on this product please see pg. 2

The specific activity of MAPKAPK5 was determined to be 440 nmol/min/mg as per activity assay protocol.

For ADP-Glo™ Assay Protocol on this product please see pg. 3

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
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 Assay Buffer I** (Catalog #: K01-09)
Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**Substrate** (Catalog #: H31-58)
HSP27tide synthetic peptide substrate (RRLNRQLSVA-amide) diluted in 25mM Tris-HCl buffer (pH 7.5) to a final concentration of 1mg/ml.

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

**[33P]-ATP Specific Activity (SA) (cpm/pmol)**
Specific activity [SA] = cpm for 5 µl [33P]-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 [33P]-ATP in cpm/pmol)*[Reaction time in min]*[Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]
ADP-Glo™ Activity Assay Protocol

**Reaction Components**

**MAPKAP5 Kinase Enzyme System**
(Protege, Catalog #: V4166)
- MAPKAP5, Active, 10µg (0.1µg/µl)
- HSP27 tide, 1ml (1mg/ml)
- Reaction Buffer A (5X), 1.5ml
- DTT (0.1M), 25µl

**ADP-Glo™ Kinase Assay Kit**
(Protege, Catalog #: V9101)
- Ultra Pure ATP solution, 10 mM (0.5ml)
- ADP solution, 10 mM (0.5ml)
- ADP-Glo™ Reagent (5ml)
- Kinase Detection Buffer (10ml)
- Kinase Detection Substrate (Lyophilized)

**Reaction Buffer A (5X)**
200mM Tris-HCl, pH 7.5, 100mM MgCl₂ and 0.5 mg/ml BSA.

**Assay Protocol**

The MAPKAP5 assay is performed using the MAPKAP5 Kinase Enzyme System (Protege; Catalog #: V4166) and ADP-Glo™ Kinase Assay kit (Protege; Catalog #: V9101). The MAPKAP5 reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

*Step 1.* Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.

*Step 2.* Thaw the components of MAPKAP5 Enzyme System, ADP and ATP on ice.

*Step 3.* Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH₂O.

*Step 4.* Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH₂O.

*Step 5.* Prepare diluted MAPKAP5 in 1X Buffer (diluted from 2X buffer) 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 MAPKAP5 for optimal results).

*Step 6.* In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1. 10µl of diluted Active MAPKAP5

Component 2. 5µl of 1mg/ml stock solution of substrate

Component 3. 5µl of 2X Buffer

*Step 7.* Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.

*Step 8.* At the same time as the MAPKAP5 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.

*Step 9.* Initiate the MAPKAP5 reactions by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.

*Step 10.* Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

*Step 11.* Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

*Step 12.* Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® plate reader (Protege; Cat# E7031).

*Step 13.* Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see Kinase Enzyme Systems Protocol at: [http://www.promega.com/KESProtocol](http://www.promega.com/KESProtocol)

**Kinase Specific Activity (SA) (nmol/min/mg)**

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(\text{ADP (step 6)} - \text{ADP (step 7)}) \text{ in nmol} / (\text{Reaction time in min}) \times (\text{Enzyme amount in mg})
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