

Recombinant Human Active MAPKAPK2 (46 - 381)

Certificate of Analysis

Catalog Number: 3705-KS

Lot Number: 1471719

Specifications and Use

- Source** ♦ Recombinant human MAPKAPK2 (amino acids 46-381) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_032960.
- Molecular Mass** ♦ The approximate molecular weight is 68 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
- Formulation** ♦ Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol.
- Size** ♦ 10 µg.
- Concentration** ♦ 0.1 µg/µL.
- Activity** ♦ The specific activity of MAPKAPK2 was determined to be 315 nmol/min/mg using a synthetic peptide substrate (RRLNRQLSVA-amide) (see activity assay protocol).
- Storage** ♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature.
♦ **Avoid repeated freeze-thaw cycles.**

MAPKAPK2

MAPKAPK2 (MAPKAP kinase 2) is a Ser/Thr protein kinase which is regulated via direct phosphorylation by p38 MAP kinase (1). In conjunction with p38 MAP kinase, MAPKAPK2 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 27 (HSP27) has been shown to be one of the substrates of MAPKAPK2 *in vivo*.

References

1. Stokoe, D. *et al.* (1993) *Biochem. J.* **296**:843.

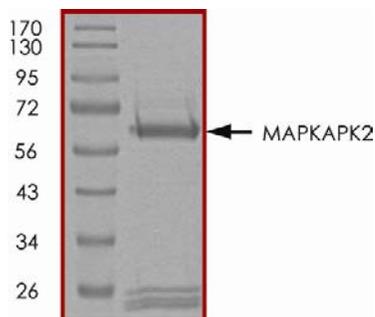


Figure 1: SDS-PAGE and Coomassie stain. The approximate molecular weight is 68 kDa and the purity is > 90%.

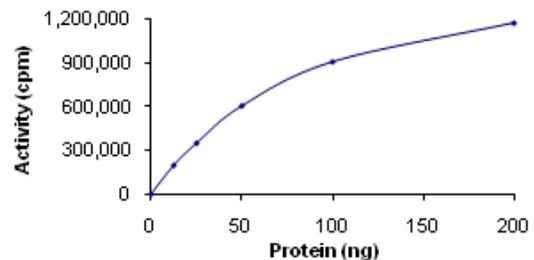


Figure 2: Enzymatic assay results. The specific activity of MAPKAPK2 was determined to be 315 nmol/min/mg as per activity assay protocol (on reverse).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active MAPKAPK2 (0.1 µg/µL) diluted with Kinase Dilution Buffer III and assayed as outlined in Figure 2.
Note: These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA.
Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer III, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/µL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- **[³³P]-ATP Assay Cocktail** - Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [³³P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer III.
- **Substrate** - HSP27-tide synthetic peptide substrate (RRLNRQLSVA-amide) diluted in distilled or deionized water to a final concentration of 1 mg/mL.

Assay Procedure

1. Thaw the [³³P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active MAPKAPK2, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active MAPKAPK2	10 µL
Substrate (1 mg/mL Stock Solution)	5 µL
Distilled H ₂ O	5 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5 µL [³³P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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.

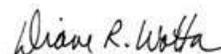
Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

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)

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

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

Diane Wotta



Quality & Regulatory Affairs