

YSK4 (MAP3K19), Active

Recombinant human protein expressed in Sf9 cells

Catalog # Y06-11G

Lot # Q2477-9

Product Description

Recombinant human YSK4 (MAP3K19) (941-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The protein accession number is [NM_025052](#).

Gene Aliases

RCK; MAP3K19

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.

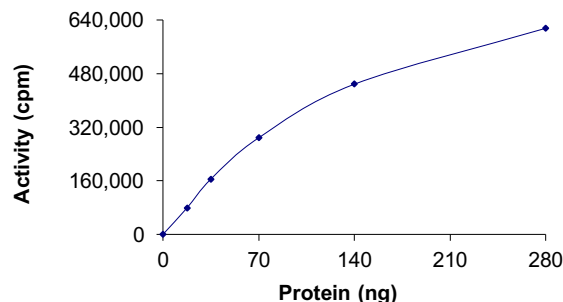
Scientific Background

YSK4 (MAP3K19, RCK) belongs to STE Ser/Thr protein kinase family and STE20 subfamily. The YSK4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat (1). The important paralog is MAP3K1 gene. The RCK ligand-binding domains form an octameric gating ring (2) in the large-conductance Ca²⁺-activated K⁺ channels (3). The YSK4 kinase function has not been made clearly yet.

References

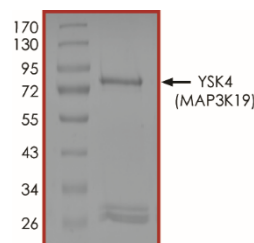
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- Ye S. *et al*: Crystal Structures of a Ligand-free MthK Gating Ring: Insights into the Ligand Gating Mechanism of K⁺ Channels. *Cell* (2006).
- Santarelli, L.C. *et al*: Three methionine residues located within the regulator of conductance for K⁺ (RCK) domains confer oxidative sensitivity to large-conductance Ca²⁺-activated K⁺ channels. *Physiol. (Lond.)* (2006).

Specific Activity



The specific activity of YSK4 (MAP3K19) was determined to be **44 nmol/min/mg** as per activity assay protocol.

Purity



The purity of YSK4 (MAP3K19) was determined to be **>80%** by densitometry, approx. MW **83 kDa**.

YSK4 (MAP3K19), Active

Recombinant human protein expressed in Sf9 cells

Catalog # Y06-11G

Specific Activity 44 nmol/min/mg

Lot # Q2477-9

Purity >80%

Concentration 0.05 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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: Y06-11G)

Active YSK4 (MAP3K19) (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 YSK4 (MAP3K19) 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 50ng/µl BSA solution.

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.

[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 II (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 II (Catalog #: K02-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: M42-51N)

MBP Protein diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active YSK4 (MAP3K19), 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 YSK4 (MAP3K19) (Catalog #Y06-11G)
 - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 20 µl 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 H₂O) 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 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 [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)]

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