

## GLK, Active

Recombinant human protein expressed in Sf9 cells

**Catalog # M25-11G**

Lot # L1969-10

### Product Description

Recombinant human GLK (1-380) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_003618](#).

### Gene Aliases

MAP4K3; MAPKKK3; MEKK3; RAB8IPL1

### 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  $-70^{\circ}\text{C}$ . 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.

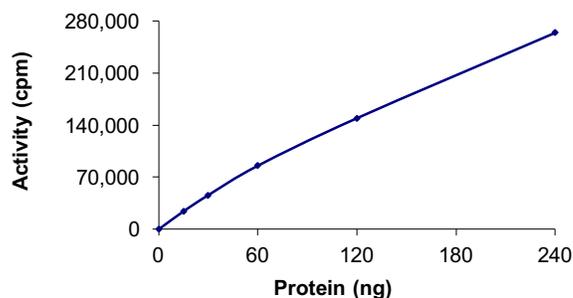
### Scientific Background

GLK is a member of the STE20 family of serine/threonine protein kinases and contains N-terminal catalytic domain and C-terminal regulatory domain. GLK is upstream of the MEK1 target and is stimulated by UV radiation and TNF- $\alpha$  and can lead to the activation of the JNK signaling pathway. GLK can directly activate PKC- $\theta$  during TCR signaling and people with systemic lupus erythematosus show considerable enhanced GLK expression and activation of PKC- $\theta$  and the kinase IKK in T cells (1). GLK can act as a pro-apoptotic kinase which orchestrates activation of BAX via the concerted posttranscriptional modulation of PUMA, BAD, and BIM (2).

### References

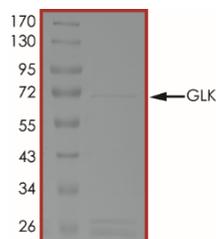
1. Chuang, H C. et al: The kinase GLK controls autoimmunity and NF- $\kappa$ B signaling by activating the kinase PKC- $\theta$  in T cells. *Nat Immunol.* 2011 Oct 9;12(11):1113-8.
2. Lam, D. et al: MAP4K3 modulates cell death via the post-transcriptional regulation of BH3-only proteins. *Proc Natl Acad Sci U S A.* 2009 Jul 21;106(29):11978-83.

### Specific Activity



The specific activity of GLK was determined to be **79 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of GLK was determined to be **>70%** by densitometry. Approx. MW **70 kDa**.

## GLK, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	M25-11G
Specific Activity	79 nmol/min/mg
Specific Lot #	L1969-10
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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 #: M25-11G)

Active GLK (0.05µg/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 GLK 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 MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>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 #: C01-58)

PKA Substrate peptide (CGRTGRRNSI-amide) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active GLK 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 GLK (Catalog #M25-11G)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C01-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 µl [<sup>33</sup>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 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 H<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>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 <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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