SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
M25-11G -05	5 µg
M25-11G -10	10 µg

GLK, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M25-11G

Lot # F696-1

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; MAPKKKK3; MEKKK3; 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°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.

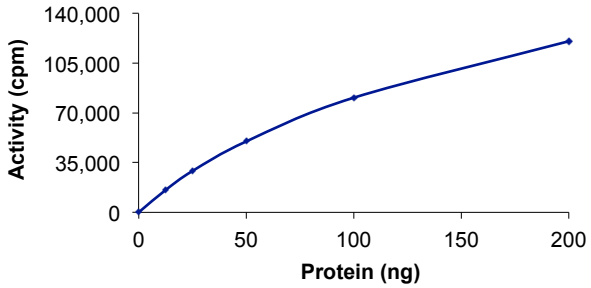
Scientific Background

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 MEKK1 target and is stimulated by UV radiation and TNF-α and can lead to the activation of the JNK signaling pathway. GLK can directly activate PKC-□ during TCR signaling and people with systemic lupus erythematosus show considerable enhanced GLK expression and activation of PKC-□ 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

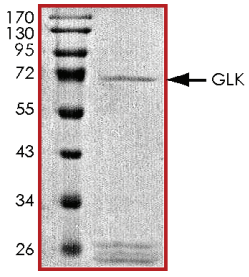
1. Chuang, H C. et al: The kinase GLK controls autoimmunity and NF-□B signaling by activating the kinase PKC-□ in T cells. Nat Immunol. 2011 Oct 9;12(11):1113-8.
2. Lam, D. et al: MAP4K3 modulates cell death via the post-transcriptional regulation of BH3-only proteins. Proc Natl Acad Sci U S A. 2009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

Specific Activity



The specific activity of GLK was determined to be **92 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 Number	M25-11G
Specific Activity	92 nmol/min/mg
Specific Lot Number	F696-1
Purity	>70%
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

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 $MgC1_2$, 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 #: C01-58)

PKA Substrate peptide (CGRTGRRNSI-amide) diluted in distilled H_2O 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 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_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 5 μl [^{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 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_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 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)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com