

## GCK, Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # M24-10G**

Lot # B2118-3

### Product Description

Recombinant full-length human GCK was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_004579](#).

### Gene Aliases

MAP4K2, BL44, RAB8IP

### 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.

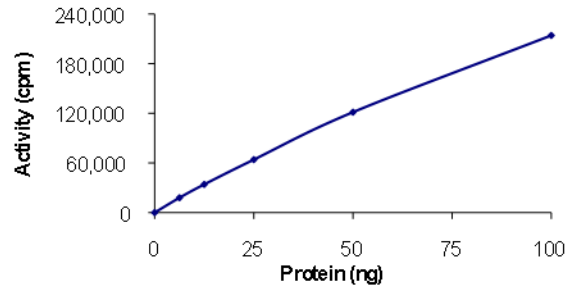
### Scientific Background

GCK is a serine/threonine protein kinase that is activated by TNF $\alpha$  and interacts with the TNF receptor-associated factor 2 (TRAF2). GCK is upstream of MAP kinases and particularly activates the SAPK pathway (1). Although GCK is found in many tissues, its expression in lymphoid follicles is restricted to the cells of germinal centre, where it may participate in B-cell differentiation. A mouse protein Rab8ip that has high homology to GCK can interact with the vesicular transport protein Rab8. Based on its interaction with Rab8, it is postulated that Rab8ip/GCK may modulate secretion in response to stress stimuli (2).

### References

1. Pombo C M, et al: Activation of the SAPK pathway by the human STE20 homologue germinal centre kinase. *Nature* 377: 750-754, 1995.
2. Ren M, et al: In its active form, the GTP-binding protein rab8 interacts with a stress-activated protein kinase. *Proc. Nat. Acad. Sci.* 93: 5151-5155, 1996.

### Specific Activity



The specific activity of GCK was determined to be **246 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of GCK was determined to be **>90%** by densitometry, approx. MW **~125kDa**.

## GCK, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	M24-10G
Specific Activity	246 nmol/min/mg
Lot #	B2118-3
Purity	>90%
Concentration	0.1 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.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: M24-10G)

Active GCK (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 GCK 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 #: M42-51N)

Myelin Basic Protein (MBP) substrate 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 GCK, 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 GCK (Catalog #M24-10G)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
  - 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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