

## GRK5 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPG03

Lot No. B170-2  
5 µg 0.1 µg/µl

### Background:

GRK5 is a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family (1). It phosphorylates the activated forms of G protein-coupled receptors thus initiating their deactivation. GRK5 plays a role in regulating the motility of polymorphonuclear leukocytes (PMNs). Desensitization of G protein-coupled receptors regulates the number of polymorphonuclear leukocytes (PMNs), as well as their motility and ability to stop upon contact with pathogens or target cells, and this desensitization is mediated by GRK5 (2).

### Product Description:

Recombinant full-length human GRK5 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM\_005308.

### Gene Aliases:

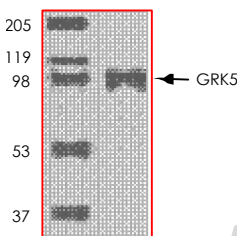
GPRK5

### Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

### Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 95kDa.



### Storage:

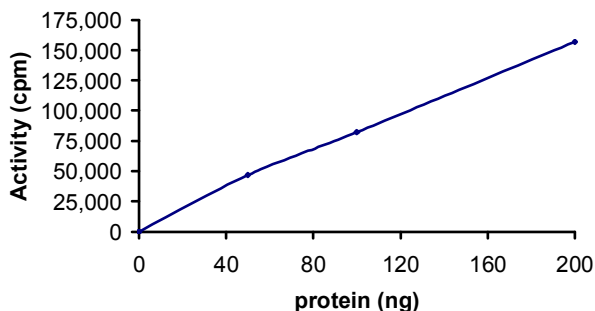
Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Stability:

Unopened vial at  $-70^{\circ}\text{C}$ , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 41 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 9  $\mu$ L of 1 mg/ml the Substrate Solution, 1  $\mu$ L of phosphatidylcholine (12.5  $\mu$ g/ $\mu$ l suspension solution), 5  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

Casein substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

- 1.Haribabu, B. et al: Identification of additional members of human G-protein-coupled receptor kinase multigene family. Proc. Nat. Acad. Sci. 90: 9398-9402, 1993.
- 2.Fan, J. et al: Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. Nature Med. 9: 315-321, 2003.

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