

## Product Specification

### GRK5, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7709-5  
 Lot #: \_\_\_\_\_  
 Aliquot size: 5 µg protein in 50 µl  
 Specific activity: 28 nmol/min/mg

### Quality Control Analysis

#### Activity assessment

GRK5 protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Casein protein in the following assay condition:

- 10 µl diluted GRK5 protein
- 9 µl Casein (1 mg/ml stock)
- 1 µl soybean phosphatidylcholine (12.5 µg/µl suspension solution)
- 5 µl [<sup>32</sup>P] ATP (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30°C and the reaction started by the addition of [<sup>32</sup>P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

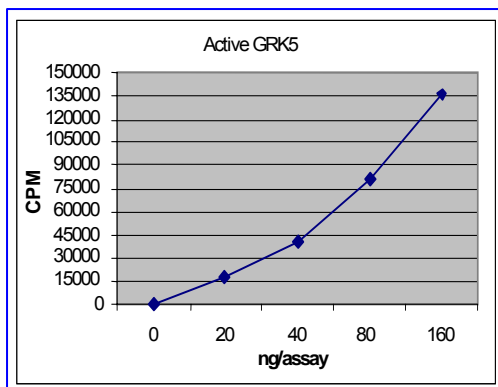


Fig. 1 GRK5 activity assay

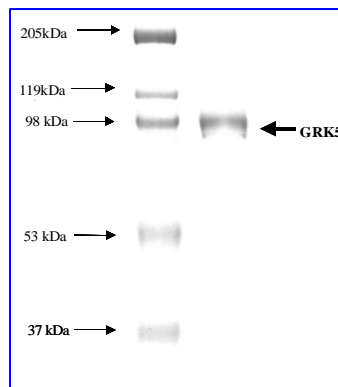


Fig. 2 GRK5 protein gel

#### Purity assessment

1.25 µg of GRK5 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the GRK5 product, and the band was at ~95 kDa (Fig. 2).

### **Product Description**

Recombinant full length human GRK5 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM\_005308.

This material is sold for research purposes only.

### Specific Activity

28 nmol phosphate incorporated into Casein per minute per mg protein at 30°C for 15 minutes using a final concentration of 50  $\mu$ M ATP (0.83  $\mu$ Ci/assay).

### Formulation

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

### Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

### Scientific Background

G protein-coupled receptor kinases (GRKs) play an important role in phosphorylating and regulating the activity of a variety of G protein-coupled receptors. Haribabu and Snyderman (1) identified GRK5 and GRK6. GRK5 is a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family. It phosphorylates the activated forms of G protein-coupled receptors thus initiating their deactivation. It has also been shown to play a role in regulating the motility of polymorphonuclear leukocytes (PMNs). Bullrich (2) used a rodent-human hybrid panel to map 2 newly identified members of the GRK family: GPRK5 and GPRK6 to 10q24-qter and 5q35, respectively. 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 GRKs (3). MIP2 induces GRK2 and GRK5 expression in PMNs through PI3KG signaling. However, lipopolysaccharide (LPS), acting through TLR4 signaling, mediated through MEK1 /MEK2, transcriptionally downregulates expression of GRK2 and GRK5 in response to MIP2, which decreases chemokine receptor desensitization and markedly augments PMN migration. Thus, LPS-activated TLR4 signaling regulates PMN migration by modulating the expression of chemokine receptors in a GRK2- and GRK5-dependent manner.

### References

1. Haribabu, B.; Snyderman, R: Identification of additional members of human G-protein-coupled receptor kinase multigene family. *Proc. Nat. Acad. Sci.* 90: 9398-9402, 1993.
2. Bullrich, F.; Druck, T.; Kunapuli, P.; Gomez, J.; Gripp, K. W.; Schlegelberger, B.; Lasota, J.; Aronson, M.; Cannizzaro, L. A.; Huebner, K.; Benovic, J. L. : Chromosomal mapping of the genes GPRK5 and GPRK6 encoding G protein-coupled receptor kinases GRK5 and GRK6. *Cytogenet. Cell Genet.* 70: 250-254, 1995.
3. Fan, J.; Malik, A. B: 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.