

GRK5, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # G03-10G

Lot # N235-3

Product Description

Recombinant full-length human GRK5 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_005308](#).

Gene Aliases

GRPK5

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.

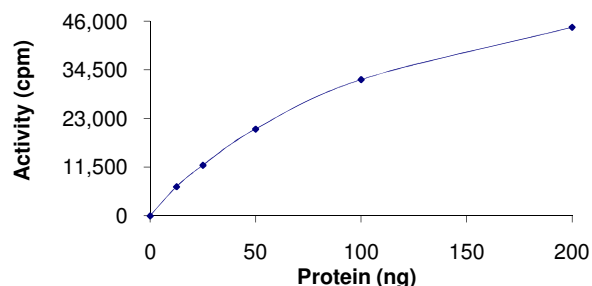
Scientific Background

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).

References

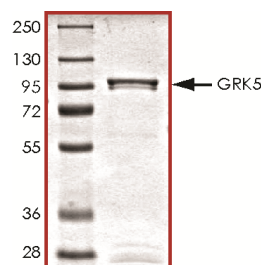
1. Haribabu, B. et al: Identification of additional members of human G-protein-coupled receptor kinase multigene family. *Proc. Nat. Acad. Sci.* 90: 9398-9402, 1993.
2. Fan, J. et al: Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression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

Specific Activity



The specific activity of GRK5 was determined to be **33 nmol/min/mg** as per activity assay protocol.

Purity



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

GRK5, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	G03-10G
Specific Activity	33 nmol/min/mg
Specific Lot Number	N235-3
Purity	>90%
Concentration	0.1µg/µl
Stability	1 yr 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 #: G03-10G)

Active GRK5 (0.1 µg/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 GRK5 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 50 ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100 µl [³³P]-ATP (1mCi/100 µl), 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 200 µl aliquots at -20°C.

Substrate (Catalog #: C03-54N)

Casein protein substrate, was diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active GRK5, 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 20 µl:
- Component 1.** 10 µl of diluted Active GRK5 (Catalog #G03-10G)
 - Component 2.** 5 µl of 1mg/ml stock solution of substrate (Catalog #C03-54N)
 - Component 3.** 1 µl phosphatidylcholine (12.5 µg/µl suspension solution)
 - Component 4.** 4 µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25 µl 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

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

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