

Certificate of Analysis

GRK5, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-714, 14-714-K, 14-714M

Parent Lot # 2398184

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, full length, human GRK5 expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 96.6% by SDS-PAGE and Coomassie blue staining. MW = 71.6kDa.

Specific Activity (Parent lot# 2398184): 204U/mg, where one unit of GRK5, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.985mg/ml of enzyme in 50mM Tris/HCl pH8.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

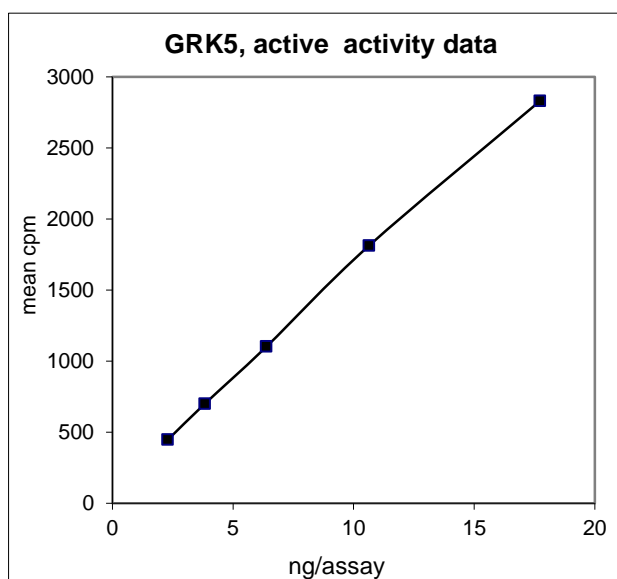
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

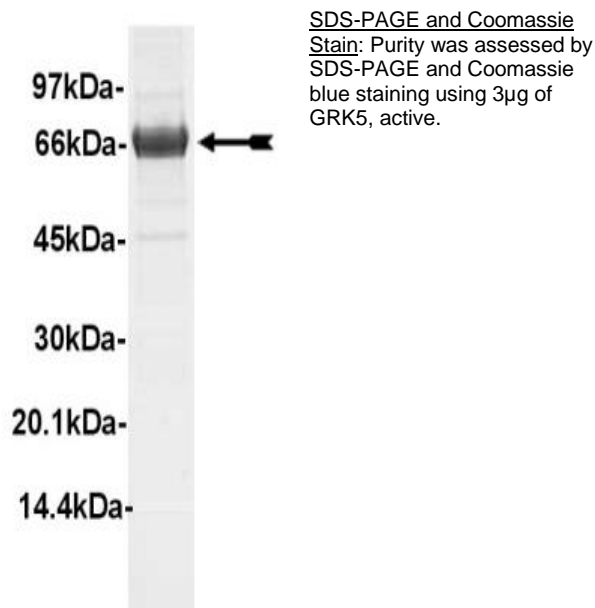
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–18ng of this lot of enzyme phosphorylated 2mg/ml casein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as GRK5 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
- 2. Casein:** Use at a final assay concentration of 2mg/ml. Make up a 20mg/ml stock. Add 2.5µl of stock per assay point.
- 3. GRK5, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–18ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **casein**.
3. Add **2.5µl (2–18ng), GRK5, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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GRK5 Sequence Information

<u>Protein</u>	Human GRK5
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human GRK5
<u>Accession number</u>	GenBank NM_005308

Recombinant GRK5 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MELENIVANT VLLKAREGGG GKRKGKSKKW
61 KEILKFPHIS QCEDLRRTID RDYCSLCKDQ PIGRLLFRQF CETRPGLECY IQFLDSVAEY
121 EVTPEKLGEG KGKEIMTKYL TPKSPVFIAQ VGQDLVSQTE EKLLQKPCKE LFSACAQSVH
181 EYLRGEPFHE YLDSMFFDRF LQWKWLERQP VTKNTFRQYR VLGKGGFGEV CACQVRATGK
241 MYACKRLEKK RIKKRKGESM ALNEKQILEK VNSQFVVNLA YAYETKDALC LVLTIMNGGD
301 LKFHIYNMGN PGFEEERALF YAAEILCGLE DLHRENTVYR DLKPENILLD DYGHIRISDL
361 GLAVKIPEGD LIRGRVGTVG YMAPEVLNNQ RYGLSPDYWG LGCLYEMIE GQSPFRGRKE
421 KVKREEVDRR VLETEEVYSH KFSEEAKSIC KMLLTKDAKQ RLGCEEGAA EVKRHPFFRN
481 MNFKRLEAGM LDPPFVDPDR AVYCKDVLDI EQFSTVKGMV LDHTDDDFYS KFSTGSVSIP
541 WQNEMIETEC FKELNVFGPN GTLPPDLNRN HPPEPPKGL LQRLFQRHQ NNSKSSPSSK
601 TSFNHHINSN HVSSNSTGSS
    
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Recombinant GRK5 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggccatgga tccggaattc atggagctgg aaaacatcgt ggccaacacg
121 gtcttgctga aagccaggga agggggcggg ggaagcgca aagggaaaag caagaagtgg
181 aaagaaatcc tgaagtccc tcacattagc cagtgtgaag acctccgaag gaccatagac
241 agagattact gcagttatg tgacaagcag ccaatcggga ggctgctttt ccggcagttt
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1741 ctccagagac tcttcaagcg gcagcatcag aacaattcca agagttcgcc cagctccaag
1801 accagtttta accaccacat aaactcaaac catgtcagct cgaactccac cggaagcagc
1861 tag

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