

Certificate of Analysis

GRK6, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-715, 14-715-K, 14-715M

Parent Lot # 1724688

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 GRK6 expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 94.9% by SDS-PAGE and Coomassie blue staining. MW = 69.8kDa.

Specific Activity (Parent lot# 1724688): 62U/mg, where one unit of GRK6, 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: 1.323mg/ml of enzyme in 50mM Tris/HCl pH8.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 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.

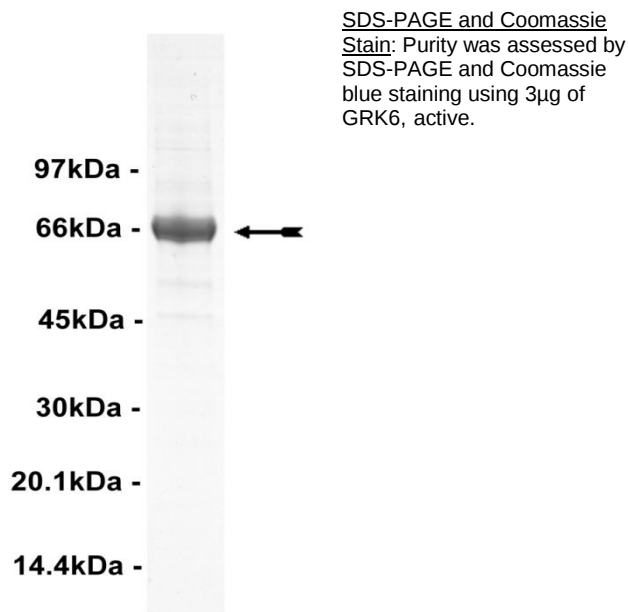
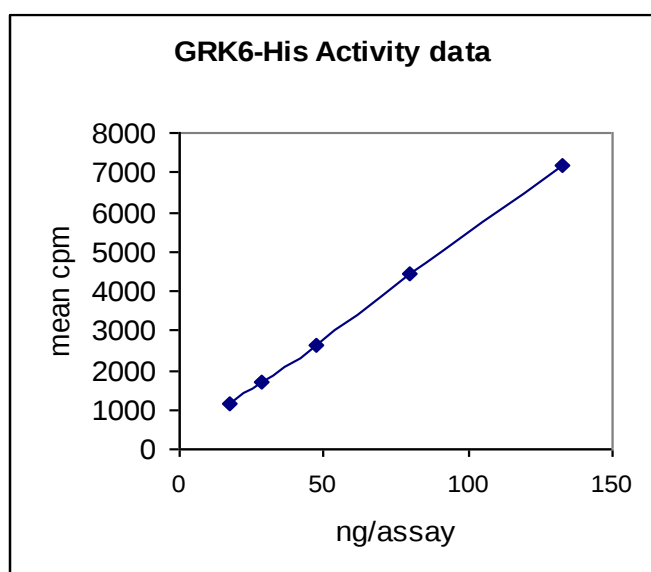
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 17–132ng 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 GRK6 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. **GRK6, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 17–132ng 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 2mg/ml **casein**.
3. Add **2.5µl (17-132ng) GRK6, 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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GRK6 Sequence Information

<u>Protein</u>	Human GRK6
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human GRK6
<u>Accession number</u>	GenBank NM_001004106

Recombinant GRK6 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MELENIVANT VLLKAREGGG GNRKGKSKKW
61 RQMLQFPHIS QCEELRLSLE RDYHSLCERQ PIGRLLFREF CATRPELSRC VAFLDGVAEY
121 EVTPDDKRKA CGRQLTQNFL SHTGPDLIPE VPRQLVTNCT QRLEQGPKCD LFQELTRLTH
181 EYLSVAPFAD YLDSIYFNRF LQWKWLERQP VTKNTFRQYR VLGKGGFGEV CACQVRATGK
241 MYACKKLEKK RIKKRKGEAM ALNEKQILEK VNSRFVVS LA YAYETKDALC LVLTLMNGGD
301 LKFHIYHMQG AGFPEARAVF YAAEICCGLE DLHRERIVYR DLKPENILLD DHGHIRISDL
361 GLAVHVPEGQ TIKGRVGTVG YMAPEVVKNE RYTFSPDWWA LGCLLYEMIA GQSPFQQRKK
421 KIKREEVERL VKEVPEEYSE RFSPQARSLC SQLLCKDPAE RLGCRGGSAR EVKEHPLFKK
481 LNFKRLGAGM LEPPFKPDPQ AIYCKDVLDI EQFSTVKGVE LEPTDQDFYQ KFATGSVPIP
541 WQNEMVETEC FQELNVFGLD GSVPPDLDWK GQPPAPPKKG LLQRLFSRQD CCGNCSDSEE
601 ELPTRL

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Recombinant GRK6 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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121 gtgctactca agggccggga aggtggcggg ggaaatcgca aaggcaaaag caagaaatgg
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1741 ctgctgcaga gactcttcag tcgccaagat tgctgtggaa actgcagcga cagcgaggaa
1801 gagctcccca cccgcctcta g

Reviewed and approved by site quality representative.

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