

Certificate of Analysis

GRK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-935, 14-935-K, 14-935M

Parent Lot # D13HP004N

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, human GRK1 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 68kDa.

Specific Activity (Parent lot# D13HP004N): 94U/mg, where one unit of GRK1, active activity is defined as 1nmol phosphate incorporated into 300µM (KKKKERLLDDRHSGLDSMKDEE) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.930mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

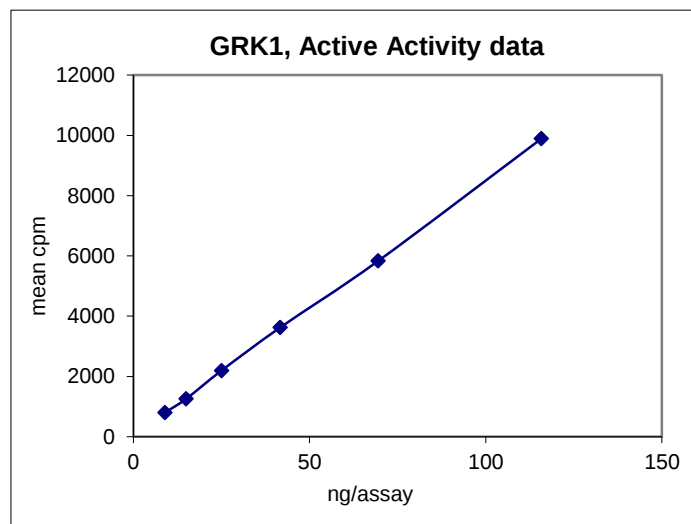
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

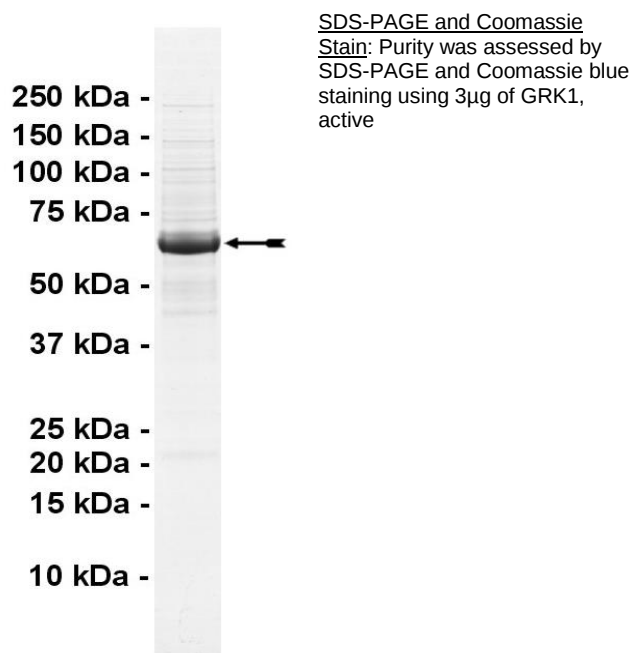
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9–193ng of this lot of enzyme phosphorylated 300µM (KKKKERLLDDRHSGLDSMKDEE) 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 GRK1 with the translated 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. **(KKKKERLLDDRHSGLDSMKDEE):** Use at a final assay concentration of 300μM. Prepare a 10mM stock and add 0.75μl of stock per assay point.
3. **10% Brij-35:** Use at a final assay concentration of 0.1% (w/v). Use 0.25μl of a 10% stock solution per assay point.
4. **Manganese Chloride:** Use at a final assay concentration of 1mM. Prepare a 100mM stock in water and use 0.25μl per assay point.
5. **Sodium orthovanadate:** Use at a final assay concentration of 1mM. Prepare a 100mM stock in water and use 0.25μl per assay point.
6. **Sodium β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock in water and use 0.025μl per assay point.
7. **GRK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9-193ng per assay point.
8. **[γ-³³P]ATP:** 2.5 x MgAc/[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 0.75μl of (KKKKERLLDDRHSGLDSMKDEE).
3. Add 0.25μl of manganese chloride
4. Add 0.25μl of 10% brij-35
5. Add 0.25μl of sodium orthovanadate
6. Add 0.025μl of sodium β-glycerophosphate
7. Add 6.15μl of dH₂O.
8. Add **2.5μl (9–193ng) GRK1, active.**
9. Add 10μl of diluted [γ-³³P]ATP mixture.
10. Incubate for 10 minutes at 30°C.
11. Stop the reaction by adding 5μl of 3% phosphoric acid.
12. Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat**.
13. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
14. Wash the filtermat once for 2 minutes with methanol.
15. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
16. 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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GRK1 Sequence Information

<u>Protein</u>	human GRK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M34 of the recombinant protein is equivalent to M1 of human GRK1
<u>Accession number</u>	GenPept NP_002920

Recombinant GRK1 amino acid sequence:

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1  MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GRRMDFGSLE TVVANSAFIA ARGSEFDGSSS
61  QPSRDKKYLA KLKLPPLSKC ESLRDSLSLE FESVCLEQPI GKKLFQQFLQ SAEKHLPALE
121 LWKDIEDYDT ADNDLQPQKA QTLAQYLDP QAKLFCSFLD EGIVAKFKEG PVEIQDGLFQ
181 PLLQATLAHL GQAPFQEYLG SLYFLRFLQW KWLEAQPMGE DWFLDFRVLG KGGFGEVSAC
241 QMKATGKLYA CKKLNKKRLK KRKG YQGAMV EKKILMKVHS RFIVSLAYAF ETKADLCLVM
301 TIMNGGDIRY HIYNVNEENP GFPEPRALFY TAQIICGLEH LHQRRIVYRD LKPENVLLDN
361 DGNVRISDLG LAVELLDGQS KTKGYAGTPG FMAPELLQGE EYDFSVDYFA LGVTLYEMIA
421 ARGPFRRARGE KVENKELKHR IISEPVKYPD KFSQASKDFC EALLEKDPEK RLGFRDETCD
481 KLAHPLFKD LNWRQLEAGM LMPPFIPDSK TVYAKDIQDV GAFSTVKGVA FDKTDEFFQ
541 EFATGNCPIP WQEEMIETGI FGELNVWRSD GQMPDDMKGI SGGSSSSSKS GMCLVS

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Recombinant GRK1 nucleotide sequence:

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1  atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61  tatttttcagg gcgccatgga tccagaattc ggtcgacgaa tggacttttg atctctcgag
121 accgctcgttg ctaactcagc ttttatcgcc gctagagggt cattcgatgg ctcatcatct
181 cagccctccc gcgacaaaaa gtacttggca aagctcaagc tgcctcctct gtccaagtgt
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1501 ctgatgccac cctttatccc cgactctaag actgtctatg cttaaagacat ccaggatgtc
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1621 gaatttgcaa ctggttaactg ccctatccct tggcaggagg agatgatcga aacaggcatc

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1681 tttggcgagc ttaacgtctg gagatctgac ggtcagatgc cagatgatat gaagggtatc
1741 agcggaggca gctcctcttc aagcaagagc ggaatgtgtc tggtcagttg a
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