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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 activity is defined as 1nmol phosphate incorporated into 300µM (KKKKERLLDDRHDSGLDSMKDEE) 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.

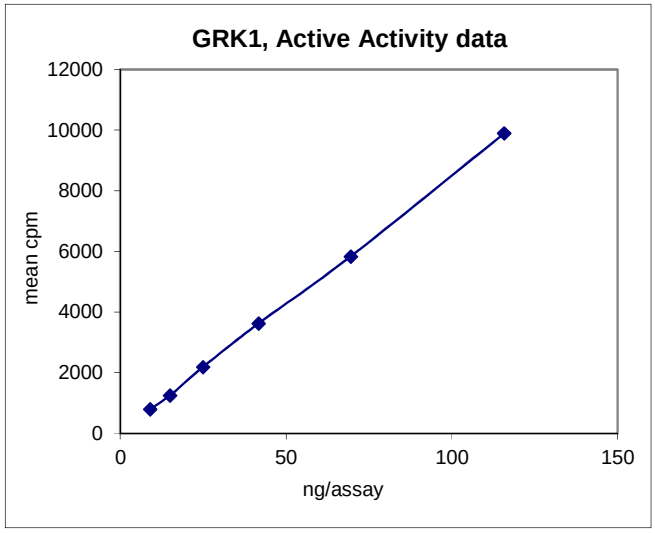
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

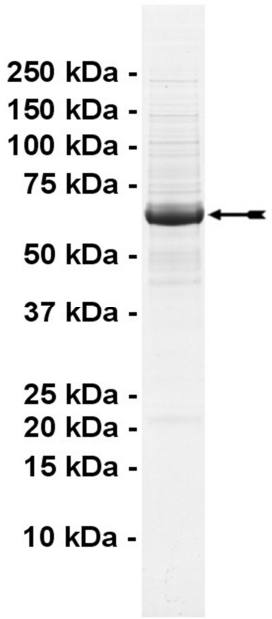
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 9–193ng of this lot of enzyme phosphorylated 300µM (KKKKERLLDDRHDSGLDSMKDEE) 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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of GRK1, active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKKERLLDDRHDSGLDSMKDEE)**: 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 orthovandate:** 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-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]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 **(KKKKERLLDDRHDSGLDSMKDEE)**.
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 dH_2O.
8. Add **2.5µl (9–193ng) GRK1, active.**
9. Add 10µl of diluted [γ-^{33}P]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

Protein human GRK1

Tags *N*-terminal 6His

Native sequence M34 of the recombinant protein is equivalent to M1 of human GRK1

Accession number GenPept NP_002920

Recombinant GRK1 amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GRRMDFGSLE TVVANSAFIA ARGSFDGSSS
 61 QPSRDKKYLA KLKLPPLSKC ESLRDSLSLE FESVCLEQPI GKKLFQQFLQ SAEKHLPALE
121 LWKDIEDYDT ADNDLQPQKA QTILAQYLDP QAKLFCSFLD EGIVAKFKEG PVEIQDGLFQ
181 PLLQATLAHL GQAPFQEYLG SLYFLRFLQW KWLEAQPMGE DWFLDFRVLG KGGFGEVSAC
241 QMKATGKLYA CKKLNKKRLK KRKGYQGAMV EKKILMKVHS RFIVSLAYAF ETKADLCLVM
301 TIMNGGDIRY HIYNVNEENP GFPEPRALFY TAQIICGLEH LHQRRIVYRD LKPENVLLDN
361 DGNVRISDLG LAVELLDGQS KTKGYAGTPG FMAPELLQGE EYDFSVDYFA LGVTLYEMIA
421 ARGPFRARGE KVENKELKHR IISEPVKYPD KFSQASKDFC EALLEKDPEK RLGFRDETCD
481 KLRAHPLFKD LNWRQLEAGM LMPPFIPDSK TVYAKDIQDV GAFSTVKGVA FDKTDTEFFQ
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 tattttcagg gcgccatgga tccagaattc ggtcgacgaa tggactttgg atctctcgag
 121 accgtcgttg ctaactcagc ttttatcgcc gctagaggtt cattcgatgg ctcatcatct
 181 cagccctccc gcgacaaaaa gtacttggca aagctcaagc tgcctcctct gtccaagtgt
 241 gaatccctga gggactccct gtctctcgag ttcgagtcag tgtgccttga acaacccatc
 301 ggtaagaaat tgtttcagca gttcctgcag tcagctgaaa aacatctgcc tgcattggag
 361 ttgtggaagg acatagaaga ctatgacaca gcggataacg atctccagcc tcagaaggct
 421 cagacgattt tggctcagta cctggacccc caagctaagc ttttctgctc tttcctggac
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 541 ccccttctgc aggcgactct cgcgcatctc ggacaggcgc ccttccagga gtacctgggc
 601 tcactctact ttctgcgctt tctgcaatgg aagtggctcg aggcgcagcc aatgggcgag
 661 gactggttcc tggacttccg cgtgctggga aagggcggct tcggagaggt gtccgcttgc
 721 cagatgaagg caaccggcaa actttatgcg tgcaagaagc tgaataagaa aagactgaag
 781 aaaaggaagg gttaccaggg agccatggta gagaagaaaa ttttgatgaa agtgcactca
 841 agattcatcg tgtcattggc ctacgcgttt gaaaccaagg ccgatttgtg cctcgtcatg
 901 acaatcatga acggcggtga tatcaggtat cacatttaca atgtcaacga ggaaaacccc
 961 ggttttccag aacccagagc cctgttctac actgctcaga tcatctgtgg acttgagcac
1021 ttgcatcagc gcaggatcgt ctacagggat ttgaaacctg aaaacgtcct cctggataac
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1261 gctagaggcc cgtttagagc acgcggagaa aaggttgaga acaaggaact caagcataga
1321 atcatcagcg agcctgtgaa atatccagac aagttctccc aggccagcaa ggacttctgt
1381 gaggctctgc ttgagaagga ccctgagaag agattgggct tccgcgatga aacctgtgat
1441 aaactcagag ctcatcctct gtttaaggat cttaactgga ggcagctgga ggcaggcatg
1501 ctgatgccac cctttatccc cgactctaag actgtctatg ctaaagacat ccaggatgtc
1561 ggtgcattct caactgttaa gggtgttgcg ttcgacaaga ccgatacgga gttcttccag
1621 gaatttgcaa ctggtaactg 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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