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Certificate of Analysis

GRK7, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-752, 14-752-K, 14-752M
Parent Lot # 33195U

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 GRK7 expressed by baculovirus in *S*f21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 66kDa.

Specific Activity (Parent lot# 33195U): 301U/mg, where one unit of GRK7, 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: 1.91mg/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.

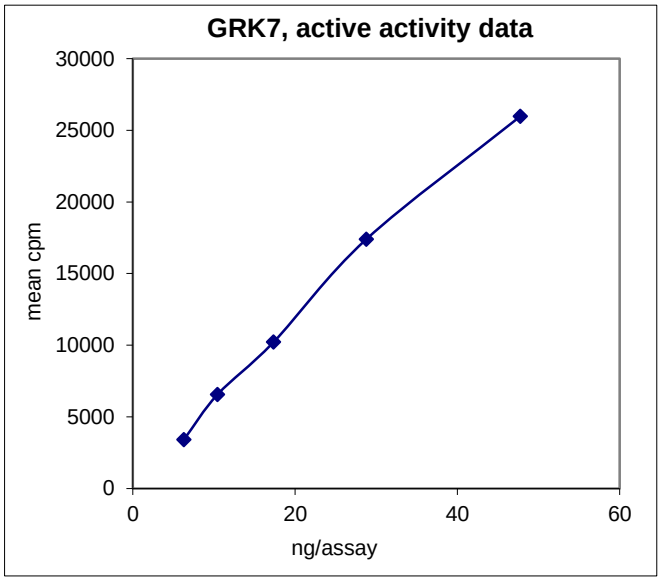
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

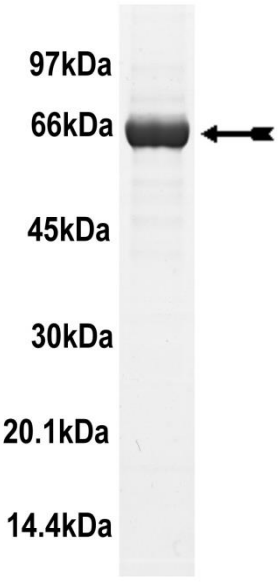
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 6–48ng 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 GRK7 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of GRK7, active.

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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. **GRK7, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–48ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[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 2.5µl of 2mg/ml **casein**.
3. Add **2.5µl (6–48ng)**, **GRK7, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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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GRK7 Sequence Information

Protein Human GRK7

Tags *N*-terminal 6His

Native sequence M31 of recombinant sequence = M1 of native human GRK7

Accession number GenBank NM_139209

Recombinant GRK7 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MVDMGALDNL IANTAYLQAR KPSDCDSKEL
  61 QRRRRSLALP GLQGCAELRQ KLSLNFHSLC EQQPIGRRLF RDFLATVPTF RKAATFLEDV
 121 QNWELAEEGP TKDSALQGLV ATCASAPAPG NPQPFLSQAV ATKCQAATTE EERVAAVTLA
 181 KAEAMAFLQE QPFKDFVTSA FYDKFLQWKL FEMQPVSDKY FTEFRVLGKG GFGEVCAVQV
 241 KNTGKMYACK KLDKKRLKKK GGEKMALLEK EILEKVSSPF IVSLAYAFES KTHLCLVMSL
 301 MNGGDLKFHI YNVGTRGLDM SRVIFYSAQI ACGMLHLHEL GIVYRDMKPE NVLLDDLGNC
 361 RLSDLGLAVE MKGGKPITQR AGTNGYMAPE ILMEKVSYSY PVDWFAMGCS IYEMVAGRTP
 421 FKDYKEKVSK EDLKQRTLQD EVKFQHDNFT EEAKDICRLF LAKKPEQRLG SREKSDDPRK
 481 HHFFKTINFP RLEAGLIEPP FVPDPSVVYA KDIAEIDDFS EVRGVEFDDK DKQFFKNFAT
 541 GAVPIAWQEE IIETGLFEEL NDPNRPTGCE EGNSSKSGVC LLL
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Recombinant GRK7 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggtggaca tgggggccct ggacaacctg
 121 atcgccaaca ccgcctacct gcaggcccgg aagccctcgg actgcgacag caaagagctg
 181 cagcggcggc ggcgtagcct ggccctgccc gggctgcagg gctgcgcgga gctccgccag
 241 aagctgtccc tgaacttcca cagcctgtgt gagcagcagc ccatcggtcg ccgcctcttc
 301 cgtgacttcc tagccacagt gcccacgttc cgcaaggcgg caaccttcct agaggacgtg
 361 cagaactggg agctggccga ggagggaccc accaaagaca gcgcgctgca ggggctggtg
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 481 gccaccaagt gccaagcagc caccactgag gaagagcgag tggctgcagt gacgctggcc
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 661 ttcactgagt tcagagtgct ggggaaaggt ggttttgggg aggtatgtgc cgtccaggtg
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1201 cctgtggact ggtttgccat gggatgcagc atttatgaaa tggttgctgg acgaacacca
1261 ttcaaagatt acaaggaaaa ggtcagtaaa gaggatctga agcaaagaac tctgcaagac
1321 gaggtcaaat tccagcatga taacttcaca gaggaagcaa aagatatttg caggctcttc
1381 ttggctaaga aaccagagca acgcttagga agcagagaaa agtctgatga tcccaggaaa
1441 catcatttct ttaaaacgat caactttcct cgcctggaag ctggcctaat tgaacccca
1501 tttgtgccag acccttcagt ggtttatgcc aaagacatcg ctgaaattga tgatttctct
1561 gaggttcggg gggtggaatt tgatgacaaa gataagcagt tcttcaaaaa ctttgcgaca
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1621 ggtgctgttc ctatagcatg gcaggaagaa attatagaaa cgggactgtt tgaggaactg
1681 aatgacccca acagacctac gggttgtgag gagggtaatt catccaagtc tggcgtgtgt
1741 ttgttattgt aa
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Reviewed and approved by site quality representative.