

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 Sf21 insect cells. Purified using Ni²⁺/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.

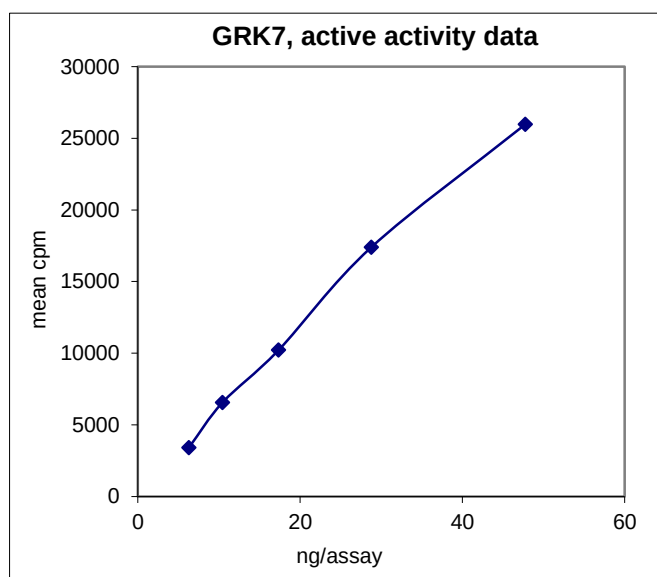
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

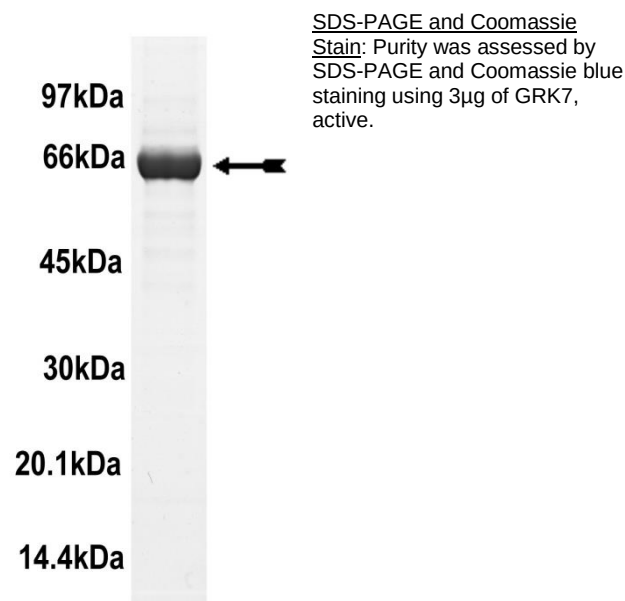
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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.



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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 -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 (6–48ng), GRK7, 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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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 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MVDMGALDNL IANTAYLQAR KPSDCDSKEL
61 QRRRRSLALP GLQGCAELRQ KLSLNFHSLC EQQPIGRRLF RDFLATVPTF RKAATFLEDV
121 QNWELAEELP TKDSALQGLV ATCASAPAPG NPQPFLSQAV ATKCAATTE EERVAAVTLA
181 KAEAMAFLQE QPFKDFVTSY FYDKFLQWKL FEMQPVSDKY FTEFRVLGKG GFGEVCAVQV
241 KNTGKMYACK KLDKKRLKKK GGEKMALLEK EILEKVSSPF IVSLAYAFES KTHLCLVMSL
301 MNGGDLKFHI YNVGTRGLDM SRVIFYSAQI ACGMLHLHEL GIVYRDMKPE NVLLDDLGNL
361 RLSDLGLAVE MKGGKPITQR AGTNGYMAPE ILMEKVSYSY PVDWFAMGCS IYEMVAGRTP
421 FKDYKEKVSF EDLKQRTLQD EVKFQHDNFT EEAKDICRLF LAKKPEQRLG SREKSDDPRK
481 HHFFKTINFP RLEAGLIEPP FVPDPSVVYA KDIAEIDDFS EVRGVEFDDK DKQFFKNFAT
541 GAVPIAWQEE ILETGLFEEL 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 tggggggccct ggacaacctg
121 atcgccaaca ccgcctacct gcaggcccg gaggccctcg actgcgacag caaagagctg
181 cagcggcggc ggcttagcct ggccctgcc gggctgcagg gctgcgcgga gctccgccag
241 aagctgtccc tgaacttcca cagcctgtgt gagcagcagc ccatcggtcg ccgcctcttc
301 cgtgacttcc tagccacagt gcccacgttc cgcaaggcgg caaccttcct agaggacgtg
361 cagaactggg agctggccga ggagggaccc accaaagaca gcgcgctgca ggggctgggtg
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541 aaggctgagg ccatggcttt cttgcaagag cagcccttta aggatttcgt gaccagcgcc
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1261 ttcaaagatt acaaggaaaa ggtcagtaaa gaggatctga agcaaagaac tctgcaagac
1321 gaggtcaaat tccagcatga taacttcaca gaggaagcaa aagatatttg caggctcttc
1381 ttggctaaga aaccagagca acgcttagga agcagagaaa agtctgatga tcccaggaaa
1441 catcatctct ttaaaacgat caacttcctc cgcctggaag ctggcctaata tgaaccccca
1501 tttgtgccag acccttcagt ggtttatgcc aaagacatcg ctgaaattga tgatttctct
1561 gaggttcggg ggggtggaatt tgatgacaaa gataagcagt tcttcaaaaa ctttgcgaca

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1621 ggtgctgttc ctatagcatg gcaggaagaa attatagaaa cgggactggt tgaggaactg
1681 aatgacccca acagacctac gggttgtgag gagggtaatt catccaagtc tggcgtgtgt
1741 ttgttattgt aa
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