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Certificate of Analysis

GRK2, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-965, 14-965-K, 14-965M
Parent Lot # D15HP015N

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 GRK2 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 82kDa.

Specific Activity (Parent lot# D15HP015N): 71U/mg, where one unit of GRK2, 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: 3.50mg/ml of enzyme in 50mM Tris/HCl pH7.5, 100mM NaCl, 0.5mM EDTA, 0.05% Triton X-100, 50% glycerol, 4mM DTT. Frozen solution.

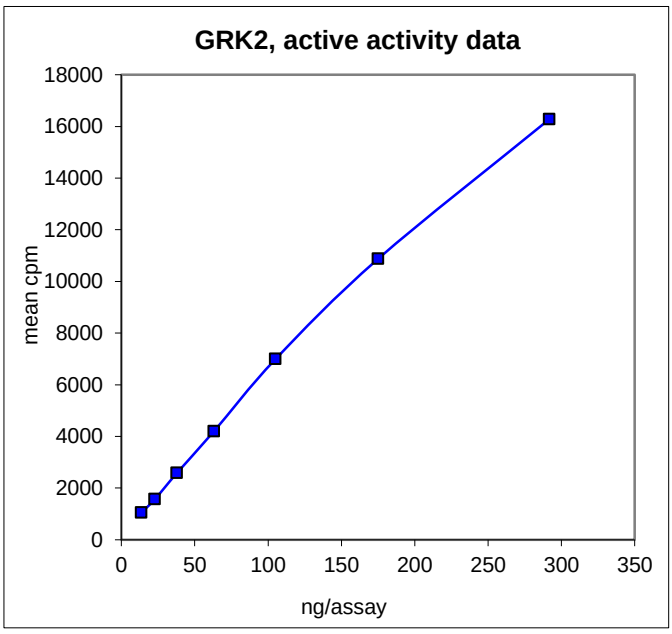
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

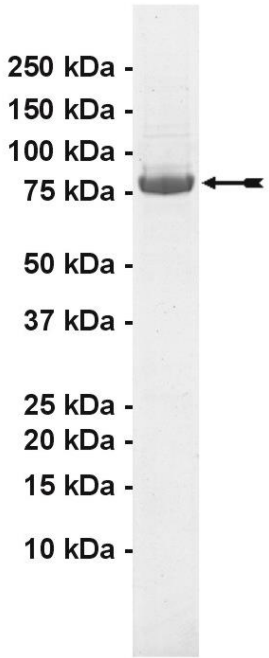
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 14–292ng 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 GRK2 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 GRK2, 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. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
3. **GRK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 14–292ng per assay point.
4. **[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 5µl of dH_2O.
3. Add 2.5µl of casein.
4. Add **2.5µl (14–292ng) GRK2, active.**
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, and dry.
11. Transfer the dried 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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GRK2, active Sequence Information

Protein	Human GRK2
Tags	*N*-terminal 6His
Native sequence	M16 of the recombinant protein is equivalent to M1 of human GRK2
Accession number	GenBank NM_001619.3

Recombinant GRK2 amino acid sequence:

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  1 MHHHHHHEFK GLRRQMADLE AVLADVSYLM AMEKSKATPA ARASKKILLP EPSIRSVMQK
 61 YLEDRGEVTF EKIFSQKLGY LLFRDFCLNH LEEARPLVEF YEEIKKYEKL ETEEERVARS
121 REIFDSYIMK ELLACSHPFS KSATEHVQGH LGKKQVPPDL FQPYIEEICQ NLRGDVFQKF
181 IESDKFTRFC QWKNVELNIH LTMNDFSVHR IIGRGGFGEV YGCRKADTGK MYAMKCLDKK
241 RIKMKQGETL ALNERIMLSL VSTGDCPFIV CMSYAFHTPD KLSFILDLMN GGDLHYHLSQ
301 HGVFSEADMR FYAAEIILGL EHMHNRFVVY RDLKPANILL DEHGHVRISD LGLACDFSKK
361 KPHASVGTHG YMAPEVLQKG VAYDSSADWF SLGCMLFKLL RGHSPFRQHK TKDKHEIDRM
421 TLTMAVELPD SFSPELRSLL EGLLQRDVNR RLGCLGRGAQ EVKESPFFRS LDWQMVFLQK
481 YPPPLIPPRG EVNAADAFDI GSFDEEDTKG IKLLDSDQEL YRNFPLTISE RWQQEVAETV
541 FDTINAETDR LEARKKAKNK QLGHEEDYAL GKDCIMHGYM SKMGNPFLTQ WQRRYFYLFP
601 NRLEWRGEGE APQSLLTMEE IQSVEETQIK ERKCLLLKIR GGKQFILQCD SDPELVQWKK
661 ELRDAYREAQ QLVQRVPKMK NKPRSPVVEL SKVPLVQRGS ANGL
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Recombinant GRK2 nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcaaa ggcctacgtc gacagatggc ggacctggag
  61 gcggtgctgg ccgacgtgag ctacctgatg gccatggaga agagcaaggc cacgccggcc
 121 gcgcgcgcca gcaagaagat actgctgccc gagcccagca tccgcagtgt catgcagaag
 181 tacctggagg accggggcga ggtgaccttt gagaagatct tttcccagaa gctggggtac
 241 ctgctcttcc gagacttctg cctgaaccac ctggaggagg ccaggccctt ggtggaattc
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 361 cgggagatct tcgactcata catcatgaag gagctgctgg cctgctcgca tcccttctcg
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 721 cgcatcaaga tgaagcaggg ggagaccctg gccctgaacg agcgcatcat gctctcgctc
 781 gtcagcactg gggactgccc attcattgtc tgcatgtcat acgcgttcca cacgccagac
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1261 acgctgacga tggccgtgga gctgcccgac tccttctccc ctgaactacg ctccctgctg
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1501 ggctccttcg atgaggagga cacaaaagga atcaagttac tggacagtga tcaggagctc
1561 taccgcaact tccccctcac catctcggag cggtggcagc aggaggtggc agagactgtc
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1621 ttcgacacca tcaacgctga gacagaccgg ctggaggctc gcaagaaagc caagaacaag
1681 cagctgggcc atgaggaaga ctacgccctg ggcaaggact gcatcatgca tggctacatg
1741 tccaagatgg gcaacccctt cctgacccag tggcagcggc ggtacttcta cctgttcccc
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2041 aacaagccgc gctcgcccgt ggtggagctg agcaaggtgc cgctggtcca gcgcggcagt
2101 gccaacggcc tctga
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Reviewed and approved by site quality representative.