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Certificate of Analysis

PrKX, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-677, 14-677-K, 14-677M
Parent Lot # 32835U

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 PrKX expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 92% by SDS-PAGE and Coomassie blue staining. MW = 44.7kDa.

Specific Activity (Parent lot# 32835U): 1015U/mg, where one unit of PrKX activity is defined as 1nmol phosphate incorporated into PAKtide (RRRLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.944mg/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.

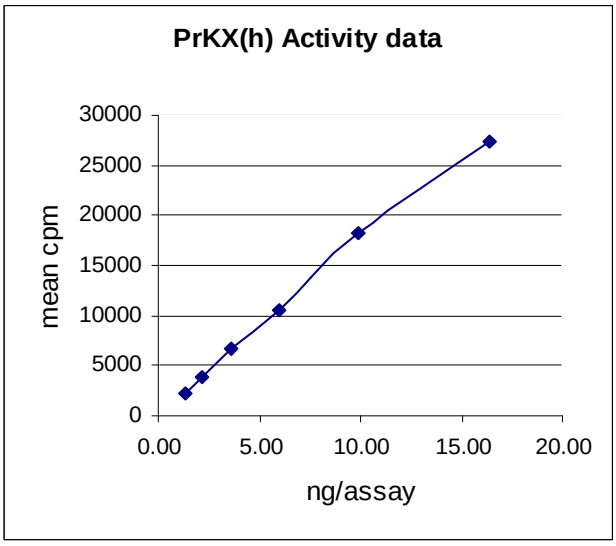
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

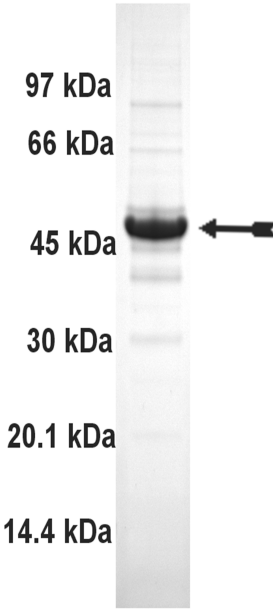
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.3–16.4ng of this lot of enzyme phosphorylated 200µM PAKtide (RRRLSFAEPG) 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 PrKX 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 PrKX, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **PAKtide (RRRLSFAEPG):** Use at a final assay concentration of 200µM. Prepare 2mM stock. Use 2.5µl of stock per assay point.
3. **PrKX, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.3–16.4ng 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 **PAKtide (RRRLSFAEPG)**.
3. Add **2.5µl (1.3–16.4ng) PrKX, 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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PrKX Sequence Information

Protein human PrKX

Tags *N*-Terminal 6His

Native sequence M31 of the recombinant sequence is equivalent to M1 of native human PrKX

Accession number GenBank NM_005044

Recombinant PrKX amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MEAPGLAQAA AAESDSRKVA EETPDGAPAL
  61 CPSPEALSPE PPVYSLQDFD TLATVGTGTF GRVHLVKEKT AKHFFALKVM SIPDVIRLKQ
 121 EQHVHNEKSV LKEVSHPFLI RLFWTWHDER FLYMLMEYVP GGELFSYLRN RGRFSSTTGL
 181 FYSAEIICAI EYLHSKEIVY RDLKPENILL DRDGHIKLTD FGFAKKLVDR TWTLCGTPEY
 241 LAPEVIQSKG HGRAVDWWAL GILIFEMLSG FPPFFDDNPF GIYQKILAGK IDFPRHLDFH
 301 VKDLIKKLLV VDRTRRLGNM KNGANDVKHH RWFRSVDWEA VPQRKLKPPI VPKIAGDGDT
 361 SNFETYPEND WDTAAPVPQK DLEIFKNF
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Recombinant PrKX nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggaggcgc ccgggctggc ccaggcggcc
 121 gcggcggaga gcgactcccg caaggtggcg gaggagaccc ccgacggggc gcccgcgctc
 181 tgccccagcc ctgaggcgct gtcgccggag ccgcctgtgt acagcctgca ggactttgac
 241 acgctggcca ccgtgggcac tgggacgttc gggcgggtgc acctggtgaa ggagaagaca
 301 gccaagcatt tcttcgccct caaggtgatg agcattcctg acgtcatccg cctaaagcag
 361 gagcaacacg tacacaatga gaagtctgtc ctgaaggaag tcagccaccc gttcctcatc
 421 aggctgttct ggacgtggca tgacgagcgc ttcctctaca tgctcatgga gtacgtgccg
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1021 gttccgcaga gaaaactgaa gcctcccatc gtgcccaaga tagctggtga cggcgacact
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1141 gatttagaaa tcttcaagaa tttctga
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Reviewed and approved by site quality representative.