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Certificate of Analysis

PRAK, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-334, 14-334-K, 14-334M
Parent Lot # 25789U

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, full-length PRAK expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 79% by SDS-PAGE and Coomassie blue staining. MW = 55kDa.

Specific Activity (Parent lot# 25789U): 230U/mg, where one unit of PRAK, active activity is defined as 1nmol phosphate incorporated into 30µM PRAK substrate peptide (KKLRRTLSVA) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 4.16mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

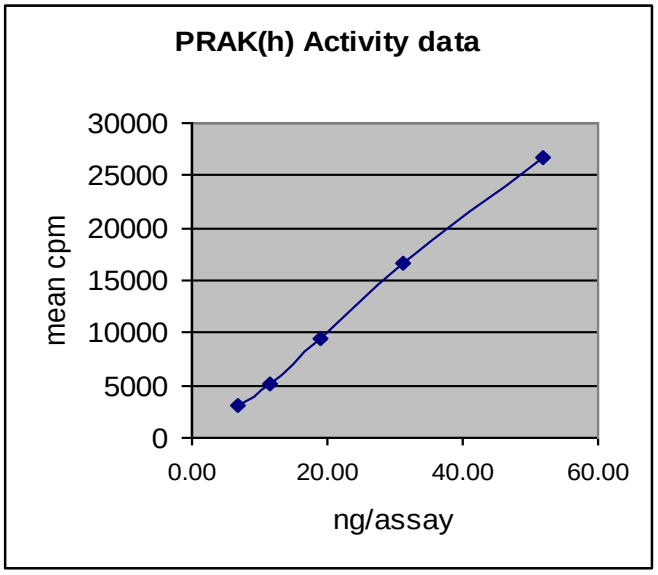
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

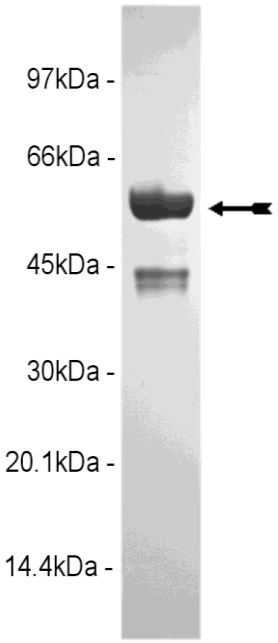
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7–52ng of this lot of enzyme phosphorylated 30µM (KKLRRTLSVA) 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 product identity as PRAK 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 PRAK, active.

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Kinase Assay Protocol

Stock Solutions:

1. **20 x Reaction Buffer:** 1M sodium-β-glycerophosphate pH7.5, 2mM EGTA.
2. **(KKLRRTLSVA):** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.
3. **PRAK, active:** Dilute with 50mM 1M sodium-β-glycerophosphate pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–52ng 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:

1. Add 1.25µl of 20 x reaction buffer to a microcentrifuge tube.
2. Add 2.5µl of **(KKLRRTLSVA),** per assay point.
3. Add **2.5µl (7–52ng) of PRAK active**.
4. Add 8.75µl 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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PRAK Sequence Information

Protein human PRAK

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human PRAK

Accession number EMBL AF032437

Recombinant PRAK amino acid sequence:

```
   1 MHHHHHHMSE ESDMDKAIKE TSILEEYSIN WTQKLGAGIS GPVRVCVKKS TQERFALKIL
  61 LDRPKARNEV RLHMMCATHP NIVQIIEVFA NSVQFPHESS PRARLLIVME MMEGGELFHR
 121 ISQHRHFTEK QASQVTKQIA LALRHCHLLN IAHRDLKPEN LLFKDNSLDA PVKLCDFGFA
 181 KIDQGDLMTP QFTPYYVAPQ VLEAQRRHQK EKSGIIPTSP TPYTYNKSCD LWSLGVIIYV
 241 MLCGYPPFYS KHHSRTIPKD MRRKIMTGSF EFPEEEWSQI SEMAKDVVRK LLKVKPEERL
 301 TIEGVLDHPW LNSTEALDNV LPSAQLMMDK AVVAGIQQAH AEQLANMRIQ DLKVSLKPLH
 361 SVNNPILRKR KLLGTKPKDS VYIHDHENGA EDSNVALEKL RDVIAQCILP QAGENEDEKL
 421 NEVMQEAWKY NRECKLLRDT LQSFSWNGRG FTDKVDRLKL AEIVKQVIEE QTTSHESQ
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Recombinant PRAK nucleotide sequence:

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   1 atgcaccatc accatcacca tatgtcggag gagagcgaca tggacaaagc catcaaggaa
  61 acttccattt tagaagaata cagtatcaat tggactcaga agctgggagc tggaattagt
 121 ggtccagtta gagtctgtgt aaagaaatct actcaagaac ggtttgcgct gaaaattctt
 181 cttgatcgtc caaaagctag aaatgaggta cgtctgcaca tgatgtgtgc cacacaccca
 241 aacatagttc agattattga agtgtttgct aacagtgtcc agtttcccca tgagtccagc
 301 cctagggccc gactcttaat tgtaatggag atgatggaag ggggagagct atttcacaga
 361 atcagccagc accggcactt tacagagaag caagccagcc aagtaacaaa gcagatagct
 421 ttggctctgc ggcactgtca cttgttaaac attgcgcaca gagacctcaa gcctgaaaat
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1321 ctgcagagct tcagctggaa tggtcgtgga ttcacagata aagtagatcg actaaaactg
1381 gcagaaattg tgaagcaggt gatagaagag caaaccacgt cccacgaatc ccaataa
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Reviewed and approved by site quality representative.

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