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Certificate of Analysis

PRAK, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-335, 14-335-K, 14-335M
Parent Lot # 1917434

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 PRAK expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 94.4% by SDS-PAGE and Coomassie blue staining. MW = 55kDa.

Specific Activity (Parent lot# 1917434): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with SAPK2a, active (cat# 14-251).

Formulation: 2.924mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Storage and Stability: On receipt of material store at -20°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.

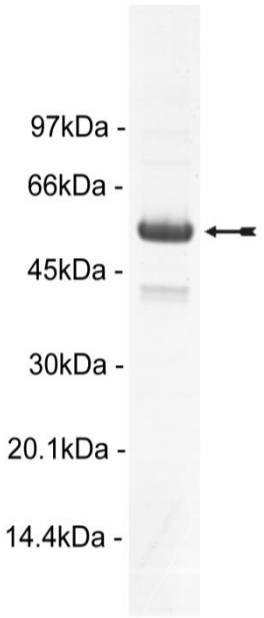
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4µM unactive PRAK was activated using SAPK2a (cat# 14-251) at a final concentration of 2U/ml, diluted 10–20 fold and the increased activity against PRAKtide (KKLRRTLSVA) measured. The activation and assay is described on page two. Results of this assay are shown below

Active SAPK2a	Unactive PRAK	Mean cpm	Comments
None	5.4µg	5	Background
50mU	5.4µg	6559	Kinase activity

MS Tryptic Fingerprint: Confirmed 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, unactive.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Activation Buffer:** 125mM Tris/HCl pH7.5, 0.5mM EGTA, 0.5% 2-mercaptoethanol.
2. **4 x PRAK assay buffer:** 1M Na-β-glycerophosphate pH7.5, 2mM EGTA.
3. **SAPK2a dilution buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1% 2-mercaptoethnaol, 1mg/ml BSA.
4. **PRAK dilution buffer:** 50mM Na-β-glycerophosphate pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
5. **PRAK, unactive:** Use at a final assay concentration of 4µM (0.216mg/ml). Prepare a 1.08mg/ml stock and add 5µl of stock per assay point.
6. **SAPK2a, active (Catalogue# 14-251):** Use at a final assay concentration of 2U/ml. Prepare a 500mU stock and add 2.5µl of stock per assay point.
7. **5 x Magnesium/ATP Cocktail:** 50mM MgAc, 0.5mM ATP.
8. **[γ-^{33}P]ATP:** 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.)
9. **PRAKtide (KKLRRTLSVA):** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of PRAK by SAPK2a*

1. Add 5µl of activation buffer to a microcentrifuge tube.
2. Add 2.5µl (50mU) of **SAPK2a, active**.
3. Add **5µl** of **unactive PRAK**.
4. Add 7.5µl of dH_2O.
5. Add 5µl of Magnesium/ATP cocktail.
6. Incubate for 60 minutes at 30°C.
7. Stop the reaction by diluting 10 and 20-fold in PRAK dilution buffer and store on ice.

Stage Two: *Phosphorylation of PRAKtide by PRAK*

1. Add 6.25µl of assay buffer to a microcentrifuge tube.
2. Add 2.5µl of **PRAKtide** stock solution.
3. Add **2.5µl** of **Stage One** reaction product.
4. Add 3.75µl of dH_2O.
5. Add 10µl of the diluted [γ-^{33}P] ATP.
6. Incubate for 10 minutes at 30°C.
7. Slowly transfer 20µl onto the centre of a 2cm x 2cm **P81** paper square.
8. Wash assay squares twice for 5 minutes with 50mM phosphoric acid.
9. Wash assay squares once with acetone for 2 minutes.
10. Transfer assay squares to scintillation vials and add 1ml scintillation cocktail.
11. Read in scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all appropriate 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. Protein is R291E with respect to this accession. This conflict is reported in GenBank AI300977, AL556217 and BI495728.

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:

```
   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
 481 ctgcttttta aggataactc tttggatgcc ccagtgaagt tgtgtgactt tggatttgcc
 541 aagattgacc aaggtgactt gatgacaccc cagttcaccc cttattatgt agcaccccag
 601 gtactggagg cgcaaagaag gcatcagaag gagaaatctg gcatcatacc tacctcaccg
 661 acgccctaca cttacaacaa gagctgtgac ttgtggtccc taggggtgat tatctatgtg
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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.