

Certificate of Analysis

PASK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-701, 14-701-K, 14-701M

Parent Lot # D12AP008N

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 PASK, amino acids 995–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 60.9% by SDS-PAGE and Coomassie blue staining. MW = 41.5kDa.

Specific Activity (Parent lot# D12AP008N): 1211U/mg, where one unit of PASK, active activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.748mg/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.

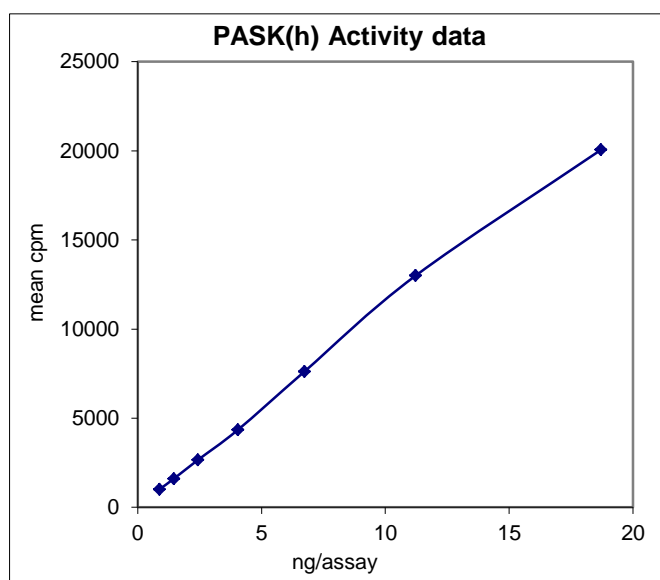
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

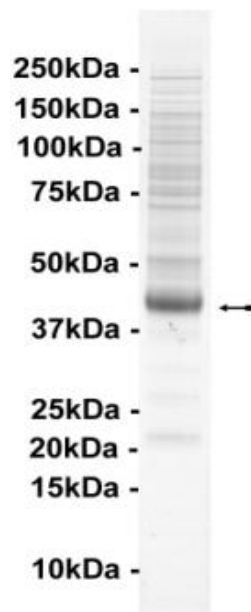
Quality Control Testing

Kinase Assay: 0.9–18.7ng of this lot of enzyme phosphorylated 250µM (KKLNRTLSFAEPG) 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 PASK 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 PASK, active.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
- 2. KKLNRTL^SFAEPG:** Use at a final concentration of 250µM. Make up a 2.5mM stock. Use 2.5µl of stock per assay point.
- 3. PASK, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.9–18.7ng 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 (KKLNRTL^SFAEPG).
3. Add **2.5µl (0.9–18.7ng), PASK 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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PASK Sequence Information

| | |
|--------------------------------|---|
| <u>Protein</u> | Human PASK |
| <u>Tags</u> | N-terminal 6His |
| <u>Native sequence</u> | Y37 of recombinant sequence is equivalent to Y995 of human PASK |
| <u>Accession number</u> | GenBank NM_015148 |

Recombinant PASK amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQYSQK YSTMSPLGSG AFGFVWTAVD
61 KEKNKEVVVK FIKKEKVLED CWIEDPKLGK VTLEIAILSR VEHANIIVKL DIFENQGFQ
121 LVMEKHGSG DLFAFIDRHP RLDEPLASYI FRQLVSAVGY LRLKDIHRD IKDENIVIAE
181 DFTIKLIDFG SAAYLERGKL FYTFCGTIEY CAPEVLMGNP YRGPPELWWS LGVTLYTLVF
241 EENPFCELEE TVEAAIHPPY LVSKEMLSLV SGLLQVPER RTTLEKLVTD PWVTQPVNLA
301 DYTWEVFRV NKPESGVLSA ASLEMGNRSL SDVAQAQELC GGPVPGAPN GQGCLHPGDP
361 RLLTS
  
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Recombinant PASK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaata ctcccaaaag
121 tacagtacca tgagcccgtc gggcagtgagg gccttcggct tcgtgtggac tgctgtggac
181 aaggaaaaaa acaaggagggt ggtggtgaag tttattaaga aggagaagggt cttggaggat
241 tgttgattg aggatcccaa acttgggaaa gttactttag agatcgcaat tctatccagg
301 gtggagcacg ccaatatcat caaggtattg gatataattg aaaaccaagg gttcttccag
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421 aggtgcttag agcccctggc gagctacatc ttccgacaac tagtgtcagc agtgggatac
481 ctgctcttga aggacatcat ccaccgtgac atcaaggatg agaacatcgt gatcgccgag
541 gacttcacaa tcaagctgat agactttggc tcggccgcct acttggaaag gggaaaatta
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1081 cgtctgctga ccagctaa
  
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