

Certificate of Analysis

ASK1, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-606, 14-606-K, 14-606M

Parent Lot # 28412U

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 GST and C-terminal 6His-tagged, recombinant, human ASK1 amino acids 649–946, expressed in *E.coli* cells. Purified using Ni²⁺/NTA agarose. Purity 72% by SDS-PAGE and Coomassie blue staining. MW = 61.5kDa.

Specific Activity (Parent lot# 28412U): 52U/mg, where one unit of ASK1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.8mg/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.

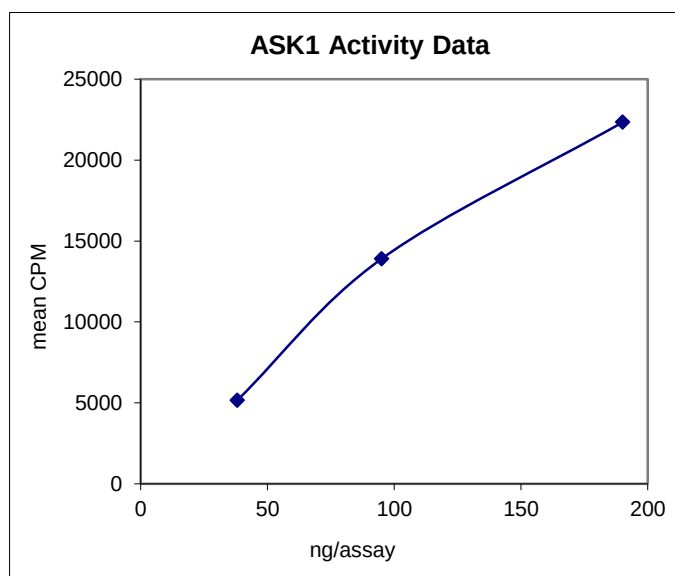
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

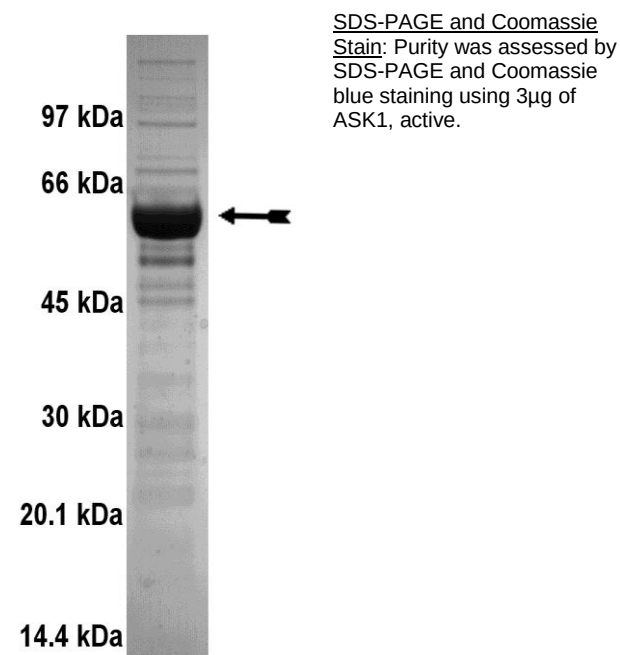
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 39–190ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) 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 ASK1 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
- 3. ASK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 39–190ng 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (39–190ng) ASK1, 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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ASK1 Sequence Information

<u>Protein</u>	human ASK1
<u>Tags</u>	N-terminal GST, C-terminal 6His
<u>Native sequence</u>	M234 of the recombinant protein is equivalent to M649 of human ASK1
<u>Accession number</u>	GenBank NM_005923

Recombinant ASK1 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMa  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFP  GRQMVNTITE
241 EKGRSTEEGD  CESDLLEYDY  EYDENGDRVV  LGKGTYGIVY  AGRDLSNQVR  IAIKEIPERD
301 SRYSQLPHEE  IALHKHLKHK  NIVQYLGFSF  ENGFIKIFME  QVPGGSLSAL  LRSKWGPKLD
361 NEQTIGFYTK  QILEGLKYLH  DNQIVHRDIK  GDNVLINTYS  GVLKISDFGT  SKRLAGINPC
421 TETFTGTLOQ  MAPEIIDKGP  RYGKAADIW  SLGCTIIEMA  TGKPPFYELG  EPQAAMFKVG
481 MFKVHPEIPE  SMSAEAKAFI  LKCFEPDPDK  RACANDLLVD  EFLKVSSKKK  KHHHHHH

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Recombinant ASK1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcattctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattg
181  ggtgatgcta  aattaacaca  gtctatggcc  atcatagctt  atatagtcga  caagcacaca
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1441  atgttttaaag  tccaccctga  gatcccagag  tccatgtctg  cagaggccaa  ggcattcata
1501  ctgaaatgtt  ttgaaccaga  tcctgacaag  agagcctgtg  ctaacgactt  gcttgttgat
1561  gagtttttaa  aagtttcaag  caaaaagaaa  aagcatcacc  atcaccatca  ctaa

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