

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ASK1 [670 – 950]

Enzyme description:- ASK1 [670 - 950]

Clone number:- DU 4710

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 58,398.88 daltons

Average Mass 58,436.46 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.27

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

Myelin Basic Protein Final concentration: 0.3 mg/ml

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Clone Data Sheet

ASK1 [670 - 950]

<u>Protein</u>	ASK1 [670 - 950]
<u>Clone number</u>	DU 4710
<u>Species</u>	Human
<u>Accession number</u>	NM_005923
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSLLEYDYEYDENGDR VVLGKGTYGIVYAGRDL SNQVRIAIKEI PERDSRY SQPLHEEIALHKHL KHKNIVQYLGFSFSENGFIKIFMEQVPGGSL SALLRSKWGPLKDNEQTIG FYTKQILEGLKYLHDNQIVHRDIKGDNLINTYSGVLKISDFGTSKRLA GINPCTETFTGTLQYMAPEIIDKGPRGYGKAADIWSLGCTI IEMATGKP PFYELGEPQAAMFKVGMFKVHPEI PESMSAEAKAFILKCFEPDPDKRAC ANDLLVDEF LKVSSKKKKKTQPK
<u>Native sequence</u>	Amino acids L670 – K950 (end T1374) of human ASK1. Residue L232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Sal</i> I sites of pGEX 6P-1

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Nucleotide
Sequence of insert

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATAACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCAAAATTAGTTTGTTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCGTTCAGGGGCCCT
GGGATCCTTGCTGGAGTATGACTATGAATATGATGAAAATGGTGACAGA
GTCGTTTTAGGAAAAGGCACTTATGGGATAGTCTACGCAGGTCGGGACT
TGAGCAACCAAGTCAGAATTGCTATTAAGGAAATCCAGAGAGAGACAG
CAGATACTCTCAGCCCCTGCATGAAGAAATAGCATTGCATAAACACCTG
AAGCACAAAATATTGTCCAGTATCTGGGCTCTTTCAGTGAGAATGGTT
TCATTAATAATCTTCATGGAGCAGGTCCCTGGAGGAAGTCTTCTGCTCT
CCTTCGTTCCAAATGGGGTCCATTAAAAGACAATGAGCAAACAATTGGC
TTTTATACAAAGCAAATACTGGAAGGATTAATAATATCTCCATGACAATC
AGATAGTTCACCGGGACATAAAGGGTGACAATGTGTTGATTAATACCTA
CAGTGGTGTCTCAAGATCTCTGACTTCGGAACATCAAAGAGGCTTGCT
GGCATAAACCCCTGACTGAACTTTTACTGGTACCCTCCAGTATATGG
CACCAGAAATAATAGATAAAGGACCAAGAGGCTACGGAAAAGCAGCAGA
CATCTGGTCTCTGGGCTGTACAATCATTGAAATGGCCACAGGAAAACCC
CCATTTTATGAACTGGGAGAACCACAAGCAGCTATGTTCAAGGTGGGAA
TGTTTAAAGTCCACCCTGAGATCCAGAGTCCATGTCTGCAGAGGCCAA
GGCATTCACTGAAATGTTTTGAACCAGATCCTGACAAGAGAGCCTGT
GCTAACGACTTGCTTGTGATGAGTTTTTAAAAGTTTCAAGCAAAAAGA
AAAAGACACAACCTAAGtgagcggccgc