Division of Signal Tranduction 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

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Clone Data Sheet

ASK1 [670 - 950]

Protein	ASK1 [670 - 950]
Clone number	DU 4710
Species	Human
Accession number	NM_005923
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**LLEYDYEYDENGDR VVLGKGTYGIVYAGRDLSNQVRIAIKEIPERDSRYSQPLHEEIALHKHL KHKNIVQYLGSFSENGFIKIFMEQVPGGSLSALLRSKWGPLKDNEQTIG FYTKQILEGLKYLHDNQIVHRDIKGDNVLINTYSGVLKISDFGTSKRLA GINPCTETFTGTLQYMAPEIIDKGPRGYGKAADIWSLGCTIIEMATGKP PFYELGEPQAAMFKVGMFKVHPEIPESMSAEAKAFILKCFEPDPDKRAC ANDLLVDEFLKVSSKKKKTQPK**
Native sequence	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.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Sal*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCT
GGGATCC**TTGCTGGAGTATGACTATGAATATGATGAAAATGGTGACAGA**
GTCGTTTTAGGAAAAGGCACTTATGGGATAGTCTACGCAGGTCGGGACT
TGAGCAACCAAGTCAGAATTGCTATTAAGGAAATCCCAGAGAGAGACAG
CAGATACTCTCAGCCCCTGCATGAAGAAATAGCATTGCATAAACACCTG
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CATCTGGTCTCTGGGCTGTACAATCATTGAAATGGCCACAGGAAAACCC
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AAAAGACACAACCTAAGtgagcggccgc