MRC PPU REAGENTS

Standard Operating Procedure

Preparation of ASK1 [2 - 1374]

Enzyme description:- ASK1 [2 – 1374]

Clone number:- DU 4707

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 181, 792.47 daltons
Average Mass 181, 907.61 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.57

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:- -80 °C

Assay:- Standard filter binding assay

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 [2 – 1374]

Protein	ASK1 [2 – 1374]
Clone number	DU 4707
Species	Human
Accession number	NM_005923
Tags	N-terminal GST
Native sequence	Amino acids S2 – T1374 (end) of human ASK1. Residue S238 of the fusion protein is equivalent to S2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Salt*1 site of pGEX 6P-3

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Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDG
DVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDF
LSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRI
EAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD**STEADEG**
ITFSVPPFAPSGFCTIPEGGICRRGGAAAVGEGEEHQLPPPPPGSFWNVESAAAPGIGCPA
ATSSSSATRGRGSSVGGGSRRTTVAYVINEASQGQLVVAESEALQSLREACETVGATLETL
HFGKLDFGETTVLDRFYNADIAVVEMSDAFRQPSLFYHLGVRESFSMANNIILYCDTNSDS
LQSLKEIICQKNTMCTGNYTFVPYMITPHNKVYCCDSSFMKGLTELMQPNFELLLGPICLP
LVDRFIQLLKVAQASSSQYFRESILNDIRKARNLYTGKELAAELARIRQRVDNIEVLTADI
VINLLLSYRDIQDYDSIVKLVETLEKLPTFDLASHHHVKFHYAFALNRRNLPGDRAKALDI
MIPMVQSEGQVASDMYCLVGRIYKDMFLDSNFTDTESRDHGASWFKKAFESEPTLQSGINY
AVLLLAAGHQFESSFELRKVGVKLSSLLGKKGNLEKLQSYWEVGFFLGASVLANDHMRVIQ
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DIWSLGCTIIEMATGKPPFYELGEPQAAMFKVGMFKVHPEIPESMSAEAKAFILKCFEPDP
DKRACANDLLVDEFLKVSSKKKKTQPKLSALSAGSNEYLRSISLPVPVLVEDTSSSSEYGS
VSPDTELKVDPFSFKTRAKSCGERDVKGIRTLFLGIPDENFEDHSAPPSPEEKDSGFFMLR
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ATTLSKLKLELDFDSHGISQVQVVLFGFQDAVNKVLRNHNIKPHWMFALDSIIRKAVQTAI
TILVPELRPHFSLASESDTADQEDLDVEDDHEEQPSNQTVRRPQAVIEDAVATSGVSTLSS
TVSHDSQSAHRSLNVQLGRMKIETNRLLEELVRKEKELQALLHRAIEEKDQEIKHLKLKSQ
PIEIPELPVFHLNSSGTNTEDSELTDWLRVNGADEDTISRFLAEDYTLLDVLYYVTRDDLK
CLRLRGGMLCTLWKAIIDFRNKQT

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Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
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