Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of CAPZIP [2 – 412]

Enzyme description:- CAPZIP [2 - 412]

Clone number:- DU 396

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 70, 763.03 daltons
Average Mass 70, 807.13 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.25

Purity:- 90 %

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:- Substrate for MAPKAP-K2

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Clone Data Sheet

CAPZIP [2 – 412]

Protein CAPZIP [2 – 412]

Clone number DU 396

Species Mouse

Accession number AAH25872

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**EERPSETNSNVDSS
AQPSVAQLAGRFREHAAVARETPASKPTRRKPPCSLPLFPPKVELGQNG
EEKSPSGASHPPKIKVKSSPLIEKLQANLAFDPAALLPGASPKSPGLKA
IVSPFHSPPSTPSSPGIRSHPSEAEEVPVSFDQPPEGTHLPSYNKVRTR
GSIKRRPPSRRFRRSQSDCGDFRDYRAVEPSQENGAREENGDDVFASKS
KDPGSPQLNQEAMADGVEGTPWSAEKPRRRNTCNSTEKPEELVRTPEEA
NAGEKVGQNPDTASQGHPEVQAPSQTGSPEAENGCGSPREETTPGEHTD
TGKATEGTASEERVADEDRLGQKSPDANMPEEEGVVREKAPQTSSGKAE
GTTIAEPDTKQKEEAPLEPSCSPGADHAAGEITSEIQNEKAVSMDDIPI
EDTRM**

Native sequence Amino acids E2 – M412 (end) of mouse CAPZIP.
Residue E232 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*HI and *Eco*R1site of pGEX 6P-1

Nucleotide Sequence of insert

ggatccGAGGAAAGACCCTCAGAGACCAACTCCAATGTTGACAGCTCAG
CGCAGCCTTCAGTGGCCCAGCTGGCTGGGCGCTTTCGGGAGCATGCAGC
TGTGGCCAGGGAGACACCAGCCAGTAAGCCAACAAGAAGGAAACCACCT
TGCTCCCTCCCCCTGTTTCCCCCCAAGGTAGAGCTGGGCCAGAACGGTG
AGGAGAAATCACCATCCGGCGCCAGCCACCCACCTAAAATCAAGGTGAA
GAGCTCACCCCTGATCGAGAAGCTTCAGGCCAACTTAGCCTTTGACCCG
GCAGCTCTTCTGCCTGGGGCTTCACCCAAAAGTCCCGGACTCAAGGCCA
TCGTATCACCATTTCACAGTCCCCCTTCCACACCCAGTAGCCCCGGCAT
CCGATCCCACCCAAGTGAGGCAGAAGAGGTGCCTGTGAGCTTTGACCAG
CCCCCGGAAGGAACTCACCTGCCCTCTTACAATAAGGTGCGGACTAGAG
GCTCAATAAAAAGACGTCCTCCCTCGCGGCGATTCCGAAGGTCTCAGTC
GGACTGTGGGGATTTTAGAGATTACAGGGCTGTGGAGCCATCTCAGGAA
AACGGTGCCAGGGAAGAGAATGGGGATGACGTGTTTGCTAGCAAGAGCA
AGGACCCCGGGTCCCCTCAACTCAACCAGGAGGCTATGGCAGACGGGGT
GGAGGGAACTCCGTGGTCTGCAGAAAAGCCACGGAGAAGGAACACGTGT
AACAGCACAGAGAAGCCAGAGGAGCTGGTCAGGACCCCAGAGGAGGCGA
ATGCTGGAGAGAAGGTTGGACAGAATCCAGACACAGCTAGTCAGGGTCA
TCCAGAGGTCCAGGCGCCATCGCAAACCGGCAGCCCAGAGGCTGAGAAT
GGGTGCGGAAGCCCACGGGAAGAGACAACCCCGGGAGAGCATACAGACA
CTGGGAAGGCCACTGAAGGGACAGCCTCTGAGGAGAGGGTAGCAGATGA
AGATAGGCTCGGACAGAAAAGCCCAGACGCAAATATGCCTGAGGAGGAG
GGAGTGGTCAGGGAGAAAGCCCCACAAACCTCTTCTGGAAAAGCAGAAG
GTACTACTATCGCAGAGCCGGATACAAAGCAAAAGGAGGAGGCGCCTCT
GGAGCCAAGCTGTAGCCCAGGGGCTGACCATGCTGCGGGAGAGATCACC
AGTGAGATCCAGAATGAGAAAGCAGTCTCCATGGATGACATCCCCATCG
AGGATACCCGAATGtgagaattc