Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ZAP70 [1 - 619]

Enzyme description:- ZAP70 [1 – 619]

Clone number:- DU 5882

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-
Monoisotopic 73, 196.40 daltons
Average Mass 73, 243.90 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.06

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc, 5 mM $MnCl_2$

Substrate:-
Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

ZAP70 [1 - 619]

Protein	ZAP70 [1 – 619]
Clone Number	DU 5882
Species	Human
Accession number	NM_001079
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MPDPAAHLPFFYGSISRAEAE EHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIERQLNGTYA IAGGKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLR DAMVRDYVRQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSL TREEAERKLYSGAQTDGKFLLRPRKEQGTYALSLIYGKTVYHYLISQDK AGKYCIPEGTKFDTLWQLVEYLKLKADGLIYCLKEACPNSSASNASGAA APTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSPDKPRPMPMDTSVY ESPYSDPEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRMRKKQI DVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYIVRLIGVCQAEALMLV MEMAGGGPLHKFLVGKREEIPVSNVAELLHQVSMGMKYLEEKNFVHRDL AARNVLLVNRHYAKISDFGLSKALGADDSYYTARSAGKWPLKWYAPECI NFRKFSSRSDVWSYGVTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMEC PPECPPELYALMSDCWIYKWEDRPDFLTVEQRMRACYYSLASKVEGPPG STQKAEAACA**
Native sequence	Amino acids M1 – A619 (end) of human ZAP70. Residue M29 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1 site of pFastBAC HTb

Nucleotide sequence of insert

ggatccATGCCAGACCCCGCGGCGCACCTGCCCTTCTTCTAC
GGCAGCATCTCGCGTGCCGAGGCCGAGGAGCACCTGAAGCTG
GCGGGCATGGCGGACGGGCTCTTCCTGCTGCGCCAGTGCCTG
CGCTCGCTGGGCGGCTATGTGCTGTCGCTCGTGCACGATGTG
CGCTTCCACCACTTTCCCATCGAGCGCCAGCTCAACGGCACC
TACGCCATTGCCGGCGGCAAAGCGCACTGTGGACCGGCAGAG
CTCTGCGAGTTCTACTCGCGCGACCCCGACGGGCTGCCCTGC
AACCTGCGCAAGCCGTGCAACCGGCCGTCGGGCCTCGAGCCG
CAGCCGGGGGTCTTCGACTGCCTGCGAGACGCCATGGTGCGT
GACTACGTGCGCCAGACGTGGAAGCTGGAGGGCGAGGCCCTG
GAGCAGGCCATCATCAGCCAGGCCCCGCAGGTGGAGAAGCTC
ATTGCTACGACGGCCCACGAGCGGATGCCCTGGTACCACAGC
AGCCTGACGCGTGAGGAGGCCGAGCGCAAACTTTACTCTGGG
GCGCAGACCGACGGCAAGTTCCTGCTGAGGCCGCGGAAGGAG
CAGGGCACATACGCCCTGTCCCTCATCTATGGGAAGACGGTG
TACCACTACCTCATCAGCCAAGACAAGGCGGGCAAGTACTGC
ATTCCCGAGGGCACCAAGTTTGACACGCTCTGGCAGCTGGTG
GAGTATCTGAAGCTGAAGGCGGACGGGCTCATCTACTGCCTG
AAGGAGGCCTGCCCCAACAGCAGTGCCAGCAACGCCTCAGGG
GCTGCTGCTCCCACACTCCCAGCCCACCCATCCACGTTGACT
CATCCTCAGAGACGAATCGACACCCTCAACTCAGATGGATAC
ACCCCTGAGCCAGCACGCATAACGTCCCCAGACAAACCGCGG
CCGATGCCCATGGACACGAGCGTGTATGAGAGCCCCTACAGC
GACCCAGAGGAGCTCAAGGACAAGAAGCTCTTCCTGAAGCGC
GATAACCTCCTCATAGCTGACATTGAACTTGGCTGCGGCAAC
TTTGGCTCAGTGCGCCAGGGCGTGTACCGCATGCGCAAGAAG
CAGATCGACGTGGCCATCAAGGTGCTGAAGCAGGGCACGGAG
AAGGCAGACACGGAAGAGATGATGCGCGAGGCGCAGATCATG
CACCAGCTGGACAACCCCTACATCGTGCGGCTCATTGGCGTC
TGCCAGGCCGAGGCCCTCATGCTGGTCATGGAGATGGCTGGG
GGCGGGCCGCTGCACAAGTTCCTGGTCGGCAAGAGGGAGGAG
ATCCCTGTGAGCAATGTGGCCGAGCTGCTGCACCAGGTGTCC
ATGGGGATGAAGTACCTGGAGGAGAAGAACTTTGTGCACCGT
GACCTGGCGGCCCGCAACGTCCTGCTGGTTAACCGGCACTAC
GCCAAGATCAGCGACTTTGGCCTCTCCAAAGCACTGGGTGCC
GACGACAGCTACTACACTGCCCGCTCAGCAGGGAAGTGGCCG
CTCAAGTGGTACGCACCCGAATGCATCAACTTCCGCAAGTTC
TCCAGCCGCAGCGATGTCTGGAGCTATGGGGTCACCATGTGG
GAGGCCTTGTCCTACGGCCAGAAGCCCTACAAGAAGATGAAA
GGGCCGGAGGTCATGGCCTTCATCGAGCAGGGCAAGCGGATG
GAATGCCCACCAGAGTGTCCACCCGAACTGTACGCACTCATG
AGTGACTGCTGGATCTACAAGTGGGAGGATCGCCCCGACTTC

CTGACCGTGGAGCAGCGCATGCGAGCCTGTTACTACAGCCTG
GCCAGCAAGGTGGAAGGGCCCCCAGGCAGCACACAGAAGGCT
GAGGCTGCCTGTGCCtgagaattcccgggtcgactcgagcgg
ccg