

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ZAP70 [1 - 619]

<u>Enzyme description:-</u>	ZAP70 [1 – 619]
<u>Clone number:-</u>	DU 5882
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	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 MnCl₂

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

MSYYHHHHHDYDIPTTENLYFQGAMGSMPPDPAHLPPFFYGSISRAEAE
EHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIERQLNGTYA
IAGGKAHC GPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVDFCLR
DAMVRDYVRQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSL
TREEAERKLYSGAQTGKFLLRPRKEQGTYALS LIYGKTVYHYLISQDK
AGKYCIPEGTKFDTLWQLVEYLKADGLIYCLKEACPNSASNASGAA
APTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSPDKPRPMPMDTSVY
ESPYS DPEELKDKKFLKRDNLLIADIELGCGNFGSVRQGVYRMRKKQI
DVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYIVRLIGVCQAEALMLV
MEMAGGGPLHKFLVGKREEIPVSNVAELLHQVSMGMKYLEEKNFVHRDL
AARNVLLVNRHYAKISDFGLSKALGADDSYTTARSAGKWPLKWAYPECI
NFRKFSSRSDVWSYGVTMWEALSYGQKPYKKMKGPVMAFIEQGKRMEC
PPECPELYALMSDCWIYKWEDRPDFLTVEQRM RACYYSLASKVEGPPG
STQKAEACA

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*I site of pFastBAC HTb

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**Nucleotide
sequence of
insert**

ggatccATGCCAGACCCCGCGGCGCACCTGCCCTTCTTCTAC
GGCAGCATCTCGCGTGCCGAGGCCGAGGAGCACCTGAAGCTG
GCGGGCATGGCGGACGGGCTCTTCCTGCTGCGCCAGTGCCCTG
CGCTCGCTGGGCGGCTATGTGCTGTGCTCGCTCGTCACGATGTG
CGCTTCCACCACTTTCCCATCGAGCGCCAGCTCAACGGCACC
TACGCCATTGCCGGCGGCAAAGCGCACTGTGGACGGGCAGAG
CTCTGCGAGTTCTACTCGCGGACCCCGACGGGCTGCCCTGC
AACCTGCGCAAGCCGTGCAACCGGCCGTGCGGGCCTCGAGCCG
CAGCCGGGGTCTTCGACTGCCTGCGAGACGCCATGGTGCGT
GACTACGTGCGCCAGACGTGGAAGCTGGAGGGCGAGGCCCTG
GAGCAGGCCATCATCAGCCAGGCCCGCAGGTGAGAAAGCTC
ATTGCTACGACGGCCACGAGCGGATGCCCTGGTACCACAGC
AGCCTGACGCGTGAGGAGCCGAGCGCAAACCTTACTCTGGG
GCGCAGACCGACGGCAAGTTCTCTGCTGAGGCCGCGGAAGGAG
CAGGGCACATACGCCCTGTCCCTCATCTATGGGAAGACGGTG
TACCACTACCTCATCAGCCAAGACAAGGCGGGCAAGTACTGC
ATTCCCGAGGGCACCAAGTTTGACACGCTCTGGCAGCTGGTG
GAGTATCTGAAGCTGAAGGCGGACGGGCTCATCTACTGCCTG
AAGGAGGCCTGCCCAACAGCAGTGCCAGCAACGCCTCAGGG
GCTGCTGCTCCCACTCCAGCCACCCATCCACGTTGACT
CATCCTCAGAGACGAATCGACACCCTCAACTCAGATGGATAC
ACCCCTGAGCCAGCACGCATAACGTCCCCAGACAAACCGCGG
CCGATGCCCATGGACACGAGCGTGTATGAGAGCCCCTACAGC
GACCCAGAGGAGCTCAAGGACAAGAAGCTCTTCCTGAAGCGC
GATAACCTCCTCATAGCTGACATTGAACTTGGCTGCGGCAAC
TTTGGCTCAGTGCGCCAGGGCGTGTACCGCATGCGCAAGAAG
CAGATCGACGTGGCCATCAAGGTGCTGAAGCAGGGCACGGAG
AAGGCAGACACGGAAGAGATGATGCGCGAGGCGCAGATCATG
CACCAGCTGGACAACCCCTACATCGTGCGGCTCATTGGCGTC
TGCCAGGCCGAGGCCCTCATGCTGGTCATGGAGATGGCTGGG
GGCGGGCCGCTGCACAAGTTCTGGTTCGCAAGAGGGAGGAG
ATCCCTGTGAGCAATGTGGCCGAGCTGCTGCACCAGGTGTCC
ATGGGGATGAAGTACCTGGAGGAGAAGAAGCTTTGTGCACCGT
GACCTGGCGGCCCGCAACGTCTCTGCTGGTTAACCGGCACTAC
GCCAAGATCAGCGACTTTGGCCTCTCAAAGCACTGGGTGCC
GACGACAGCTACTACTGCCCCGCTCAGCAGGGAAAGTGGCCG
CTCAAGTGGTACGCACCCGAATGCATCAACTTCCGCAAGTTC
TCCAGCCGCAGCGATGTCTGGAGCTATGGGGTACCATGTGG
GAGGCCTTGTCTACGGCCAGAAGCCCTACAAGAAGATGAAA
GGGCCGAGGTGATGGCCTTCATCGAGCAGGGCAAGCGGATG
GAATGCCACACAGAGTGTCCACCCGAACTGTACGCACTCATG
AGTGACTGCTGGATCTACAAGTGGGAGGATCGCCCCGACTTC

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CTGACCGTGGAGCAGCGCATGCGAGCCTGTTACTACAGCCTG
GCCAGCAAGGTGGAAGGGCCCCAGGCAGCACACAGAAGGCT
GAGGCTGCCTGTGCCTgagaattcccgggtcgactcgagcgg
ccg