

MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of CAPZB (2 – 272)

<u>Enzyme description:-</u>	CAPZB [2 – 272]
<u>Clone number:-</u>	DU 3276
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Cobalt Agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	33, 847.77 daltons
Average Mass	33, 869.17 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.82
<u>Purity:-</u>	>80 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol	
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

CAPZB [2 – 272]

Protein CAPZB [2 – 272]

Clone number DU 3276

Species Human

Accession number NM_004930.5

Tags N-terminal His6

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSSDQQLDCALDLMRRLPQQIE
KNLSDLIDLVP^SLCEDLLSSVDQPLKIARDKVVGKDYLLCDYNRDGDSY
RSPWSNKYDPPLEDGAMP SARLRKLEVEANNAFDQYRDLYFEGGVSSVY
LWDLDHGFAGVILIKKAGDGSKKIKGCWDSIHVVEVQEKSSGRTAHYKL
TSTVMLWLQTNKSGSGTMNLGGSLTRQMEKDETVSDCSPHIANIGRLVE
DMENKIRSTLNEIYFGKTKDIVNGLRSVQTFADKSKQEALKNDLVEALK
RKQQC

Native sequence Amino acids S2 – C272 (end) of human CAPZB.
Residue S29 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage TEV Protease (ENLYFQG) residues 14 - 21

Cloning sites *Bam*H1 and *Not*1 sites of pFastBac HTb

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Nucleotide
Sequence of insert

ggatccAGTGATCAGCAGCTGGACTGTGCCTTGGACCTAATGAGGCGCC
TGCCTCCCCAGCAAATCGAGAAAACCTCAGCGACCTGATCGACCTGGT
CCCCAGTCTATGTGAGGATCTCCTGTCTTCTGTTGACCAGCCACTGAAA
ATTGCCAGAGACAAGGTGGTGGGAAAGGATTACCTTTTGTGTGACTACA
ACAGAGATGGGGACTCCTATAGGTCACCATGGAGTAACAAGTATGACCC
TCCCTTGGAGGATGGGGCCATGCCGTCAGCTCGGCTGAGAAAGCTGGAG
GTGGAAGCCAACAATGCCTTTGACCAGTATCGAGACCTGTATTTTGAAG
GTGGCGTCTCATCTGTCTACCTCTGGGATCTGGATCATGGCTTTGCTGG
AGTGATCCTCATAAAGAAGGCTGGAGATGGATCAAAGAAGATCAAAGGC
TGCTGGGATTCCATCCACGTGGTAGAAGTGCAGGAGAAATCCAGCGGTC
GCACCGCCATTACAAGTTGACCTCCACGGTGATGCTGTGGCTGCAGAC
CAACAAATCTGGCTCTGGCACCATGAACCTCGGAGGCAGCCTTACCAGA
CAGATGGAGAAGGATGAAACTGTGAGTGAAGTACTGCTCCCCACACATAGCCA
ACATCGGGCGCCTGGTAGAGGACATGGAAAATAAAATCAGAAGTACGCT
GAACGAGATCTACTTTGGAAAAACAAAGGATATCGTCAATGGGCTGAGG
TCTGTGCAGACTTTTGCAGACAAATCAAAACAAGAAGCTCTGAAGAATG
ACCTGGTGGAGGCTTTGAAGAGAAAGCAGCAATGCTaagcggccgc