Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active UBA6 [1 - 1052]

Enzyme description:- UBA6 [1 – 1052]

Clone number:- DU 11583

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Calculated molecular mass:-
Monoisotopic 122, 704.24 daltons
Average Mass 122, 782.22 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.82

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

UBA6 [1 - 1052]

Protein	UBA6 [1 – 1052]
Clone Number	DU 11583
Species	Human
Accession number	NM_018227.5
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGSGIQRPTSTSSLVAAA**MEGSEP VAAHQGEEASCSSWGTGSTNKNLPIMSTASVEIDDALYSRQRYVLGDTA MQKMAKSHVFLSGMGGLGLEIAKNLVLAGIKAVTIHDTEKCQAWDLGTN FFLSEDDVVNKRNRAEAVLKHIAELNPYVHVTSSSVPFNETTDLSFLDK YQCVVLTEMKLPLQKKINDFCRSQCPPIKFISADVHGIWSRLFCDFGDE FEVLDTTGEEPKEIFISNITQANPGIVTCLENHPHKLETGQFLTFREIN GMTGLNGSIQQITVISPFSFSIGDTTELEPYLHGGIAVQVKTPKTVFFE SLERQLKHPKCLIVDFSNPEAPLEIHTAMLALDQFQEKYSRKPNVGCQQ DSEELLKLATSISETLEEKPDVNADIVHWLSWTAQGFLSPLAAAVGGVA SQEVLKAVTGKFSPLCQWLYLEAADIVESLGKPECEEFLPRGDRYDALR ACIGDTLCQKLQNLNIFLVGCGAIGCEMLKNFALLGVGTSKEKGMITVT DPDLIEKSNLNRQFLFRPHHIQKPKSYTAADATLKINSQIKIDAHLNKV CPTTETIYNDEFYTKQDVIITALDNVEARRYVDSRCLANLRPLLDSGTM GTKGHTEVIVPHLTESYNSHRDPPEEEIPFCTLKSFPAAIEHTIQWARD KFESSFSHKPSLFNKFWQTYSSAEEVLQKIQSGHSLEGCFQVIKLLSRR PRNWSQCVELARLKFEKYFNHKALQLLHCFPLDIRLKDGSLFWQSPKRP PSPIKFDLNEPLHLSFLQNAAKLYATVYCIPFAEEDLSADALLNILSEV KIQEFKPSNKVVQTDETARKPDHVPISSEDERNAIFQLEKAILSNEATK SDLQMAVLSFEKDDDHNGHIDFITAASNLRAKMYSIEPADRFKTKRIAG KIIPAIATTTATVSGLVALEMIKVTGGYPFEAYKNCFLNLAIPIVVFTE TTEVRKTKIRNGISFTIWDRWTVHGKEDFTLLDFINAVKEKYGIEPTMV VQGVKMLYVPVMPGHAKRLKLTMHKLVKPTTEKKYVDLTVSFAPDIDGD EDLPGPPVRYYFSHDTD**
Native sequence	Amino acids M1 – D1052 (end) of human UBA6. Residue M44 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	*Not*1 site of pFastBAC HTb

Nucleotide sequence of insert

gcggccgcgATGGAAGGATCCGAGCCTGTGGCCGCCCATCAGGGGGAAG
AGGCGTCCTGTTCTTCCTGGGGGACTGGCAGCACAAATAAAAATTTGCC
CATTATGTCAACAGCATCTGTGGAAATCGATGATGCATTGTATAGTCGA
CAGAGGTACGTTCTTGGAGACACAGCAATGCAGAAGATGGCCAAGTCCC
ATGTTTTCTTAAGTGGGATGGGTGGTCTTGGTTTGGAAATTGCAAAGAA
TCTTGTTCTTGCAGGGATTAAGGCAGTTACAATTCATGATACAGAAAAA
TGCCAAGCATGGGATCTAGGAACCAACTTCTTTCTCAGTGAAGATGATG
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ACCACAGATCTCTCCTTTTTAGATAAATACCAGTGTGTAGTATTGACTG
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GAGAAGAACCAAAAGAAATTTTCATTTCAAACATAACGCAAGCAAATCC
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