

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2A

<u>Enzyme description:-</u>	UBE2A (2-152)
<u>Clone number:-</u>	DU4203
<u>Source:-</u>	human recombinant
<u>Tag:-</u>	cleaved from N-terminal GST-
<u>Purification method:-</u>	GSH-Sepharose, protease treatment
<u>Expression system:-</u>	<i>E.coli</i>
<u>Calculated molecular mass:-</u>	
Monoisotopic	18253 Da
Average Mass	18263 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	4.97
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP

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Clone Data Sheet

UBE2A

<u>Protein</u>	UBE2A (2-152)
<u>Synonyms</u>	ubiquitin-conjugating enzyme E2A (RAD6 homolog); ubiquitin-protein ligase A; ubiquitin carrier protein A; HR6A; RAD6 homolog A
<u>Clone Number</u>	DU4203
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_003327
<u>Tags</u>	cleaved from GST-
Aminoacid sequence of the purified protein	<u>GPLGSPNSRVDSTPARRRLMRDFKRLQEDPPAGVSGAPSENNIMVWNAVIFG</u> <u>PEGTPFEDGTFKLTIEFTEEYPNKPPTVRFVSKMFHPNVYADGSICLDILQN</u> <u>RWSPTYDVSSILTSIQSLLDEPNPNSPAN SQAAQLYQENKREYEKRVSAIVE</u> <u>QSWRDC</u>
Native sequence	Start Met is missing.
Protease cleavage	Prescission protease site underlined
Cloning sites	Sal1 / Not1
<u>DNA sequence of insert</u>	ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC TCGACTTCTTTTGAATATCTTGAAGAAAATATGAAGAGCATTTGTATG AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA GTCTATGGCCATCATACTTATATAGCTGACAAGCACACATGTTGGGTG GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC AATGTGCCTGGATGCGTTCCCAAATTAGTTTGTTTTAAAAACGTATTG AAGCTATCCCAAATTTGATAAGTACTTGAAATCCAGCAAGTATATAGCA TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCTGGGATCCCCGAATT CCCGGTTCGACTCCACCCCGGCTCGGCGGCCTCATGCGGGACTTCAAG AGGTTGCAGGAGGATCCTCCAGCCGGAGTCAGCGGGGCTCCGTCGAGAA CAACATAATGGTGTGGAACGCGGTCATTTTCGGGCCTGAAGGGACCCCGT TTGAGGATGGAACATTTAAACTTACAATAGAATTCCTGAAGAATATCCA AATAAACACCTACAGTTAGATTTGTCTCTAAGATGTTCCATCCAAATGT CTATGCAGATGGTAGTATATGTCGGACATACTCAGAACCCTGGAGTC CAACCTATGATGTGTCTTCCATTCTAACATCCATACAGTCTCTGTTGGAT GAACCAATCCCAATAGTCCAGCAAACAGCCAGGCTGCTCAGCTGTACCA GGAGAACAAACGGGAATATGAAAAGCGTGTCTTGCAATAGTAGAACAAA GCTGGCGTGATTGTTGAGCGGCCG