

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2A

Enzyme description:- GST-UBE2A (2-152)

Clone number:- DU4203

Source:- human recombinant

Tag:- N-terminal GST-

Purification method:- GSH-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 44648 Da

Average Mass 44676 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.62

Purity:- 90%

Enzyme storage buffer:-

50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT

Storage temperature:- -80°C

Assay:-

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

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Clone Data Sheet

GST-UBE2A

<u>Protein</u>	GST-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	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFE LGFLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAELISM LEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHK YLNQDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPO IDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSPNSR VDSTPARRRLMRDFKRLQEDPPAGVSGAPSENNIMVWNAVIFGPEGT PFEDGTFKLTIEFTTEEYPNKPPTVRFVSKMFHPNVYADGSICLDILQ NRWSPTYDVSSILTSIQSLLEPNPNSPANSQAAQLYQENKREYEKR VSAIVEQSWRDC
Native sequence	Start Met is missing.
Protease cleavage	Prescission protease site underlined
Cloning sites	Sal1 / Not1
<u>DNA sequence of insert</u>	ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC TCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAATGTTGGGTG GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC AATGTGCCTGGATGCGTTCCCAAATTAGTTTGTGTTTTAAAAACGTATTG AAGCTATCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA TGGCCTTTCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCTGGGATCCCCGAATT CCCGGTCGACTCCACCCCGGCTCGGCGGCGCCTCATGCGGACTTCAAG AGGTTGCAGGAGGATCCTCCAGCCGGAGTCAGCGGGGCTCCGTCCGAGAA CAACATAATGGTGTGGAACGCGGTCATTTTCGGGCCTGAAGGGACCCCGT TTGAGGATGGAACATTTAAACTTACAATAGAATTCCTGGAAGAATATCCA AATAAACACCTACAGTTAGATTTGTCTCTAAGATGTTCCATCCAAATGT CTATGCAGATGGTAGTATATGTCTGGACATACTTCAGAACCCTGGAGTC CAACCTATGATGTGTCTTCCATCTAACATCCATACAGTCTCTGTTGGAT GAACCAATCCCAATAGTCCAGCAAACAGCCAGGCTGCTCAGCTGTACCA

GGAGAACAAACGGGAATATGAAAAGCGTGTTTCTGCAATAGTAGAACAAA
GCTGGCGTGATTGTTGAGCGGCCGC