

*Division of Signal Transduction Therapy*

**Standard Operation Procedure**

**Preparation of His-UBE2A**

**Enzyme description:-** His-UBE2A 2-152 (end)

**Clone number:-** DU20178

**Source:-** human recombinant

**Tag:-** N-terminal His<sub>6</sub>-tag

**Purification method:-** Ni<sup>++</sup>-NTA-Sepharose

**Expression system:-** E.coli

**Calculated molecular mass:-**

Monoisotopic 21010 Da

Average Mass 21022 Da

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.4

**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**

#### **His-UBE2A**

<b><u>Protein</u></b>	His-UBE2A 2-152 (end)
<b><u>Synonyms</u></b>	ubiquitin-conjugating enzyme E2A (RAD6 homolog); ubiquitin-protein ligase A; ubiquitin carrier protein A; HR6A; RAD6 homolog A
<b><u>Clone Number</u></b>	DU20178
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: NP_003327
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag
Aminoacid sequence of the expressed protein	HHHHHSSGLVPRGSHMASMTGGQOMGRDPNSSSVD <b>STPARRRLMRDFKR</b> <b>LQEDPPAGVSGAPSENNIMVWNAVIFGPEGTPFEDGTFKLTIEFTEEYPN</b> <b>KPPTVRFVSKMFHPNVYADGSICLDILQNRWSPTYDVSSILTSIQSLLDE</b> <b>PNPNSPANSQAAQLYQENKREYEKRVSAIVEQSWRDC</b>
Native sequence	Start Met is missing
Protease cleavage	Thrombin site underlined
Cloning sites	Sal1 / Not1
<b><u>DNA sequence of insert</u></b>	<u>GTCGACTCCACCCCGGCTCGGCGGCCTCATGCGGGACTTCAAGAGGTTGC</u> AGGAGGATCCTCCAGCCGGAGTCAGCGGGGCTCCGTCCGAGAACAACATAAT GGTGTGGAACCGGTCATTTTCGGCCTGAAGGGACCCCGTTTGAGGATGGA ACATTTAAACTTACAATAGAATTCCTGAAGAATATCCAAATAAACCACCTA CAGTTAGATTTGTCTCTAAGATGTTCCATCCAAATGTCTATGCAGATGGTAG TATATGTCTGGACATACTTCAGAACCGTTGGAGTCCAACCTATGATGTGTCT TCCATTCTAACATCCATACAGTCTCTGTTGGATGAACCAATCCCAATAGTC CAGCAAACAGCCAGGCTGCTCAGCTGTACCAGGAGAACAAACGGGAATATGA AAAGCGTGTTCCTGCAATAGTAGAACAAGCTGGCGTGATTGTTGAGCGGCC <u>GC</u>