

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2W

Enzyme description:- His-UBE2W 1-162 (full length)

Clone number:- DU20190

Source:- human recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic 22189 Da

Average Mass 22204 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.32

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

Loading buffer: 50mM HEPES pH 7.5

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Clone Data Sheet

His-UBE2W

Protein His-UBE2W 1-162 (full length)

Synonyms

Clone Number DU20190

Species Human

Accession Number Protein: NP_001001481 DNA: NM_001001481.1

Tags N-terminal His-tag

Aminoacid sequence of the expressed protein **MGSSHHHHHSSGLVPRGSHMASMTGGQOMGRGSMASMQTTGRRVEVWFPKRLQKELLALQNDPPPGMTLNEKSVQNSITQWIVDMEGAPGTLYEGEKFO LLFKFSSRYPFDSPQVMFTGENIPVHPHVYSNGHICLSILTEDWSPALSV QSVCLSIISMLSSCKEKRRPPDNSFYVRTCNKNPKKTKWVYHDDTC**

Native sequence in bold

Protease cleavage Thrombin site underlined

Cloning sites BamH1 / Not1

DNA sequence of insert GGATCCATGGCGTCAATGCAGACCACAGGAAGGAGGGTGGAAGTATGGTT TCCAAAACGACTACAGAAAGAACTGTTGGCTTTGCAAAATGACCCACCTC CTGGAATGACCTTAAATGAGAAGAGTGTTCAAATTC AATTACACAGTGG ATTGTAGACATGGAAGGTGCACCAGGTACCTTATATGAAGGGGAAAAATT TCAACTTCTATTTAAATTTAGTAGTCGATATCCTTTT GACTCTCCTCAGG TCATGTTTACTGGTGAAAATATTCCTGTTTCATCCTCATGTTTATAGCAAT GGT CATATCTGTTTATCCATTCTAACAGAA GACTGGTCCCCAGCGCTCTC AGTCCAATCAGTTTGTCTTAGCATTATTAGCATGCTTTCCAGCTGCAAGG AAAAGAGACGACCACCGGATAATTC TTTTATGTGCGAACATGTAACAAG AATCCAAAGAAAACAAAATGGTGGTATCATGATGATACTTGT TGAGCGGC CGC