

*Division of Signal Transduction Therapy*

**Standard Operation Procedure**

**Preparation of His-TRIAD1**

<b><u>Enzyme description:-</u></b>	TRIAD1
<b><u>Clone number:-</u></b>	DU20432 (SC20432)
<b><u>Source:-</u></b>	Sf21 Recombinant
<b><u>Tag:-</u></b>	His <sub>6</sub> -
<b><u>Purification method:-</u></b>	Ni-Sepharose
<b><u>Expression level:-</u></b>	18mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	61150 Da
Average Mass	61188 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	5.1
<b><u>Purity:-</u></b>	80-90%
<b><u>Enzyme storage buffer:-</u></b>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	
Ub-Ligase assay with UBE1 and UBE2L3	

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**Clone Data Sheet**

**Protein name**

**Protein** His-TRIAD1;  
**Synonyms** ARIH2; ARI-2, Ariadne-2 homolog  
**Clone Number** DU20432  
**Species** Human  
**Accession Number** Protein: O95376 DNA: NM\_006321  
**Tags** His<sub>6</sub>-

Aminoacid sequence of the expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSMSVDMNSQGSDSNEEDYDPNCE  
EEEEEEEDDPGDIEDYYVGVASDVEQOGADAFDPEEYQFTCLTYKESEGA  
LNEHMTSLASVLKVSHSVAKLILVNFHWQVSEILDYKSNQAQLLVEARV  
QPNPSKHVPTSHPPHCAVCMQFVRKENLLSLACQHQFCRSCWEQHCSVL  
VKDGVGVGVSCMAQDCPLRTPEDFVFP LLPNEELREKYRRYLFRDYVESH  
YQLQLCPGADCPMVI RVQEP RARRVQCNRCNEVFCFKRQMYHAPTDCAT  
IRKWLTKCADDSETANYISAHTKDCPKCNICIEKNGGCNMQCSKCKHDF  
CWMCLGDWKTHGSEYYECSRYKENPDIVNQSQAQAREALKKYL F YFERW  
ENHNKSLQLEAQTYQRIHEKIQERVMNNLGTWIDWQYLQNAAKLLAKCRY  
TLQYTPYAYYMESGPRKLF EYQQAQLEAEIENLSWKVERADSYDRGDL  
ENQMHIAEQRRRTLLKDFHDT

Native sequence

Protease cleavage TEV underlined

Cloning sites BamHI / NotI

**DNA sequence of  
insert**

GGATCCATGTCAGTGGACATGAATAGCCAGGGGTCTGACAGCAATGAAGA  
GGACTATGACCCAAATTGTGAGGAAGAGGAAGAAGAAGAAGACGACC  
CTGGGGACATAGAGGACTATTACGTGGGAGTAGCCAGCGATGTGGAGCAG  
CAGGGGGCTGATGCCTTTGATCCCGAGGAGTACCAGTTCACCTTGCTTGAC  
CTACAAGGAATCTGAGGGTGCCCTCAATGAGCACATGACCAGCTTAGCTT  
CTGTCCTAAAGGTATCTCATTTCAGTTGCTAAACTTATATTAGTTAATTTT  
CACTGGCAAGTTTCAGAGATATTGGACAGATAACAAGTCCAATTCTGCTCA  
ACTGCTTGTTGAGGCTCGAGTTCAGCCTAATCCATCAAACATGTTCCCA  
CATCCCATCCCCCTCACCCTGTGCAGTGTGTATGCAGTTTGTGCGAAAG  
GAAAACCTACTCTCTCTGGCCTGTCAGCACCAGTTTTTGCCGCAGCTGCTG  
GGAGCAGCACTGCTCAGTTCTCGTCAAGGACGGCGTGGGCGTGGGAGTCT  
CTTGTCATGGCTCAGGACTGTCCACTCCGTACACCAGAGGACTTTGTGTTTT  
CCATTGCTTCCCAATGAAGAATTGAGAGAGAAATACAGGCGCTACCTCTT  
CAGGGACTATGTGGAGAGTCAATTACCAGCTCCAGCTGTGCCCTGGTGCAG  
ACTGCCCCATGGTTATTCGGGTACAGGAGCCTAGAGCTCGCCGAGTACAG  
TGCAATCGGTGCAACGAGGTCTTCTGTTTTCAAGTGTGTCGTCAGATGTATCA  
CGCACCCACAGACTGTGCCACAATCCGGAAATGGCTCACGAAGTGTGCAG  
ACGACTCTGAAACAGCCAACTACATTAGTGCTCACACTAAAGACTGTCCC  
AAGTGCAACATCTGCATTGAGAAGAATGGAGGCTGCAATCACATGCAATG  
CTCCAAATGTAAACACGACTTCTGCTGGATGTGTCTAGGAGATTGGAAGA  
CTCATGGCAGTGAATACTATGAGTGCAGTCGTTACAAGGAGAATCCTGAC  
ATCGTGAACCAGAGCCAACAAGCCAGGCGAGGGAAGCCCTCAAGAAGTA  
CTTATTCTACTTTGAGAGGTGGGAAAACCACAATAAAAGCTTGCAGCTAG  
AGGCACAGACATAACCAGCGGATTCACGAGAAGATTCAGGAGAGGGTCATG  
AACAACTCTGGGGACATGGATCGACTGGCAGTACCTACAGAATGCTGCCAA  
GCTCTTGGCCAAGTGTGCATACACCCTGCAATACACCTACCCATATGCAT  
ATTACATGGAGTCCGGACCCAGGAAGAAGCTGTTTGAATACCAGCAGGCT  
CAGCTGGAGGCTGAGATCGAAAACCTCTCATGGAAAGTGGAGCGTGCAGA  
CAGCTATGACAGAGGGGACTTGGAGAACCAGATGCATATAGCGGAGCAGC  
GGAGGAGAACCCTGCTGAAAGATTTCCATGACACCTAAGCGGCCGC