

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of TRIAD1 L84A, V86A, W100A

Enzyme description:- TRIAD1 1-493 L84A, V86A, W100A

Clone number:- DU22466

Source:- BL21 Recombinant

Tag:- cleaved from N-terminal GST

Purification method:- GSH-Sepharose, protease treatment

Expression level:- 0.3 mg/L

Calculated molecular mass:-

Monoisotopic 57596

Average Mass 57631

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.77

Purity:- 80%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

Division of Signal Transduction Therapy

Clone Data Sheet

Protein name TRIAD1 L84A, V86A, W100A

<u>Protein</u>	TRIAD1 1-493 L84A, V86A, W100A
<u>Synonyms</u>	ARI2, ARIH2, HT005
<u>Clone Number</u>	DU22466
<u>Species</u>	human
<u>Accession Number</u>	Protein:
<u>Tags</u>	N-terminal GST-Prescission-Protease
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLE FPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGAVLDI RYGVSRIAYSKDFFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQ GWQATFGGGDHPPKSDLEVLFGPLGSMMSVDMNSQGSDSNEEDYDPNCEEE EEEEEDDPGDIEDYYVGVASDVEQQGADAFDPEEYQFTCLTYKESEGALNE HMTSLASVAKASHSVAKLILVNFHAQVSEILDYKSNQAQLLVEARVQPNP SKHVPTSHPPHCAVCMQFVRKENLLSLACQHQFCRSCWEQHCSVLVKDGV GVGVSMAQDCPLRTPEDFVFPLLPNEELREKYRRYLFRDYVESHYQLQLC PGADCPMVIQVQEPARRVQCNRCNEVFCFKCRQMYHAPTDCAITRKWLTK CADDSETANYISAHTKDCPKCNICIEKNGGCNHMQCSKCKHDFCWMCLGDW KTHGSEYYECSRYKENPDIVNQSQQAQAREALKKYLFFERWENHNKSLQL EAQTYQRIHEKIQERVMMNLGTWIDWQYLQNAKLLAKCRYTLQYTPYAY YMESGPRKKLFEYQQAQLEAEIENLSWKVERADSYDRGDLENQMHIAEQRR RTLLKDFHDT
Native sequence	in bold
Protease cleavage	Prescission Protease site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of the insert</u>	ggatccATGTCAGTGGACATGAATAGCCAGGGGTCTGACAGCAATGAAGAG GACTATGACCCAAATTGTGAGGAAGAGGAAGAAGAAGAAGACGACCCCT GGGGACATAGAGGACTATTACGTGGGAGTAGCCAGCGATGTGGAGCAGCAG GGGGCTGATGCCTTTGATCCCGAGGAGTACCAGTTCACTTGCTTGACCTAC AAGGAATCTGAGGGTGCCCTCAATGAGCACATGACCAGCTTAGCTTCTGTC gcAAAGgcATCTCATTCACTTGTCTAACTTATATTAGTTAATTTCCACgcG CAAGTTTCAGAGATATTGGACAGATACAAGTCCAATTCTGCTCAACTGCTT GTTGAGGCTCGAGTTCAGCCTAATCCATCAAAACATGTTCCACATCCCAT CCCCCTCACCCTGTGCACTGTGTATGCAGTTTGTGCGAAAGGAAAACCTA CTCTCTCTGGCCTGTGAGCACCAGTTTTTGCCGCAGCTGCTGGGAGCAGCAC TGCTCAGTTCTCGTCAAGGACGGCGTGGGCGTGGGAGTCTCTTGATGGCT CAGGACTGTCCACTCCGTACACCAGAGGACTTTGTGTTTCCATTGCTTCCC AATGAAGAATTGAGAGAGAAATACAGGCGCTACCTCTTCAGGGACTATGTG GAGAGTCATTACCAGCTCCAGCTGTGCCCTGGTGCAGACTGCCCCATGGTT ATTCGGGTACAGGAGCCTAGAGCTCGCCGAGTACAGTGCAATCGGTGCAAC GAGGTCTTCTGTTTCAAGTGTGTCAGATGTATCACGCACCCACAGACTGT GCCACAATCCGGAAATGGCTCACGAAGTGTGCAGACGACTCTGAAACAGCC AACTACATTAGTGCTCACACTAAAGACTGTCCCAAGTGCAACATCTGCATT GAGAAGAATGGAGGCTGCAATCACATGCAATGCTCCAAATGTAAACACGAC

TTCTGCTGGATGTGTCTAGGAGATTGGAAGACTCATGGCAGTGAATACTAT
GAGTGCAGTCGTTACAAGGAGAATCCTGACATCGTGAACCAGAGCCAACAA
GCCCAGGCGAGGGAAGCCCTCAAGAAGTACTTATTCTACTTTGAGAGGTGG
GAAAACCACAATAAAAAGCTTGCAGCTAGAGGCACAGACATAACCAGCGGATT
CACGAGAAGATTCAGGAGAGGGTCATGAACAATCTGGGGACATGGATCGAC
TGGCAGTACCTACAGAATGCTGCCAAGCTCTTGGCCAAGTGTCGATACACC
CTGCAATACACCTACCCATATGCATATTACATGGAGTCCGGACCCAGGAAG
AAGCTGTTTGAATACCAGCAGGCTCAGCTGGAGGCTGAGATCGAAAACCTC
TCATGGAAAGTGGAGCGTGCAGACAGCTATGACAGAGGGGACTTGGAGAAC
CAGATGCATATAGCGGAGCAGCGGAGGAGAACCCTGCTGAAAGATTTCCAT
GACACCTAAgcggccgc