

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of ITSN1 S313A S315A [1 – 1220]

Enzyme description:- ITSN1 S313A S315A [1 - 1220]

Clone number:- DU 27301

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 222, 796.98 daltons

Average Mass 222, 936.65 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.00

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

ITSN1 S313A S315A [1 - 1220]

Protein ITSN1 S313A S315A [1 - 1220]

Clone number DU 27301

Species Human

Accession number NM_001001132.1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL
DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA
WPLQGWQATFGGGDHPKSDLEVLFGGGLGSPNSRVEMAQFPTPFGGSLD
IWAITVEERAKHDQQFHSLKPTISGFITGDQARNFFQSGLPQPVLAQIWA
LADMNDGRMDQVEFSIAMKLIKLLKQGYQLPSALPPVMKQQPVAISSAP
AFGMGGIASMPPLTAVAPVPMGSIIPVVGMSPTLVSSVPTAAVPLANGAP
PVIQPLPAFAHPAATLPKSSFSRSGPGSQLNFKLQKAQSFVAVPPVA
EWAVPQSSRLKYRQLFNSHDKTMSGHLTGPOARTILMQSSLPQAQLASIW
NLSIDIDQDGKLTAEFFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRA
GAGISVISSTSDVQRLPEEPVLEDEQQOLEKKLPVTFEDKKRENFERNL
ELEKRRQALLEQQRKEQERLAQLERAQERKERERQEQERKRQLELEKQL
EKQRELERQREERKEIERREAAKRELERQRLQLEWERNRQELLNQRNK
EQEDIVVLKAKKKTLEFELEALNDKKHQLEGLQDIRCRLTTQRQEIEST
NKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILNDQLKQVQQNSLHRD
SLVTLKRALEAKELARQHLRDQLDEVEKETRSKLQEQIDIFNNQLKELREI
HNKQQLQKQKSMEAERLKQKEQERKIIIELEKQKEEAQRAQERDKQWLEH
VQQEDEHQRPRLHEEEKLRKREESVKKKDGEKQQAQDKLGRLFHQHQ
EPAKPAVQAPWSTAEGKPLTISAQENVKVYRALLYPFESRSHDEITIQP
GDIVMVKGEWVDESQTGEPGWLGGELKGTGWFPANYAEKIPENEVPAPV
KPVTDSTAPAPKLALRETPAPLAVTSSEPSTTPNNWADFSSTWPTSTNE
KPETDNWDAWAAQPSLTVPSAGQLRQSAFTPATATGSSPSPVLGQGEKV
EGLQAQALYPWRAKKNHNLNFKNDVITVLEQQDMWWFGEVQGGKQWFPK
SYVKLISGPIRKSTSMDSGSSSESPASLKRVASPAAKPVVSGEEFIAMTY
ESSEQGDLTFFQQGDVILVTKKDGWWTGTVDKAGVFPSPNYVRLKDSGS
GTAGKTGSLGKKPEIAQVIASYTATGPEQLTLAPQLILIRKKNPGWWE
GELQARGKKRQIGWFPANYVKLLSPGTSKITPTEPPKSTALAAVCQVIGM
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DMDPSQQWCSDLHLLDMLTPTERKRQGYIHELIVTEENYVNDLQLVTEIF
QKPLMESELLTEKEVAMIFVNWKEIMCNILKALRVRKMSGEKMPVK
MIGDILSAQLPHMQPYIRFCSRQLNGAALIQQKTDEAPDFKEFVKRLAMD
PRCRGMPLSSFILKPMQRVTRYPLIKNILENTPENHPDHSKHALEKA
EELCSQVNEGVREKENSRLLEWIQAHVQCEGLSEQLVFNSVTNCLGPRKF
LHSGKLYKAKSNKELYGFLFNDLLLQITKPLGSSGTDKVFSPKSNLQY
KMYKTFIFLNEVLVKLPTDPSGDEPIFHISHIDRVYTLRAESINERTAW
QKKAASELYIETEKKKREKAYLVRSQRATGIGRLMVNVVEGIELKPCRS
HGKSNPYCEVTMGSQCHITKTIQDTLNPKNWNSCQFFIRDLEQEVLCITV
FERDQFSPDDFLGRTEIRVADIKKQDQSGKGPVTKCLLLHEVPTGEIVVRLDLQLFDEP

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Native sequence

Amino acids M1 – P1220 (end) of human ITSN1.
Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The enzyme has an S313A and S315A mutation. Residue S313 is equivalent to A550 and residue S315 is equivalent to A552 of the fusion protein.

Protease cleavage

PreScission (LEVLFQGP) residues 221 – 228.

Cloning sites

*Xho*1 and *Not*1 into *Sal*1 and *Not*1 site of pGex6P1

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Nucleotide Sequence

ATGGCTCAGTTTCCAACACCTTTTGGTGGCAGCCTGGATATCTGGCCATAACTGTAGAGGAAAGAGCGAAGCATGATCAGCAGTT
CCATAGTTTAAAGCCAATATCTGGATTCACTACTGGTATCAAGTGTAGAACTTTTTTTTCACTGGGTACCTCAACCTGTTT
TAGCACAGATATGGGCCTAGCTGACATGAATAATGATGGAAGATGGATCAAGTGGAGTTTTCCATAGCTATGAACTTATCAA
CTGAAGCTACAAGGATATCAGCTACCTCTGCACCTCCCTGTGCATGAAACAGCAACCAGTTGCTATTTCTAGCGCACCAGCATT
TGGTATGGGAGGTATCGCCAGCATGCCACCCTTACAGCTGTTGCTCCAGTGCCTCAATGGGATCCAGTTGTTGGAATGCTC
CAACCCTAGTATCTTCTGTTCCACAGCAGCTGTGCCCCCTGGCTAACGGGGCTCCCTGTTATACAACCTCTGCCTGCATTT
GCTCATCTGCAGCCACATGGCCAAAGAGTCTTCCCTTAGTAGATCTGGTCCAGGGTCCACAACATAACCTAAATACAAAAGGC
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