MRC PPU REAGENTS

Standard Operating Procedure

Preparation of ITSN1 S315A [1 – 1220]

Enzyme description:- ITSN1 S315A [1 - 1220]

Clone number:- DU 27300

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 222, 800.94 daltons
Average Mass 222, 940.60 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 S315A [1 - 1220]

Protein ITSN1 S315A [1 - 1220]

Clone number DU 27300

Species Human

Accession number NM_001001132.1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL
DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA
WPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVE**MAQFPTPFGGSLD**
IWAITVEERAKHDQQFHSLKPISGFITGDQARNFFFQSGLPQPVLAQIWA
LADMNNDGRMDQVEFSIAMKLIKLKLQGYQLPSALPPVMKQQPVAISSAP
AFGMGGIASMPPLTAVAPVPMGSIPVVGMSPTLVSSVPTAAVPPLANGAP
PVIQPLPAFAHPAATLPKSSSFSRSGPGSQLNTKLQKAQSFDVASVPPVA
EWAVPQSSRLKYRQLFNSHDKTMSGHLTGPQARTILMQSSLPQAQLASIW
NLSDIDQDGKLTAEEFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRS
GAGISVISSTSVDQRLPEEPVLEDEQQQLEKKLPVTFEDKKRENFERGNL
ELEKRRQALLEQQRKEQERLAQLERAEQERKERERQEQERKRQLELEKQL
EKQRELERQREEERRKEIERREAAKRELERQRQLEWERNRRQELLNQRNK
EQEDIVVLKAKKKTLEFELEALNDKKHQLEGKLQDIRCRLTTQRQEIEST
NKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILNDQLKQVQQNSLHRD
SLVTLKRALEAKELARQHLRDQLDEVEKETRSKLQEIDIFNNQLKELREI
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VQQEDEHQRPRKLHEEEKLKREESVKKKDGEEKGKQEAQDKLGRLFHQHQ
EPAKPAVQAPWSTAEKGPLTISAQENVKVVYYRALYPFESRSHDEITIQP
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KPVTDSTSAPAPKLALRETPAPLAVTSSEPSTTPNNWADFSSTWPTSTNE
KPETDNWDAWAAQPSLTVPSAGQLRQRSAFTPATATGSSPSPVLGQGEKV
EGLQAQALYPWRAKKDNHLNFNKNDVITVLEQQDMWWFGEVQGQKGWFPK
SYVKLISGPIRKSTSMDSGSSESPASLKRVASPAAKPVVSGEEFIAMYTY
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GTAGKTGSLGKKPEIAQVIASYTATGPEQLTLAPGQLILIRKKNPGGWWE
GELQARGKKRQIGWFPANYVKLLSPGTSKITPTEPPKSTALAAVCQVIGM
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DMDPSQQWCSDLHLLDMLTPTERKRQGYIHELIVTEENYVNDLQLVTEIF
QKPLMESELLTEKEVAMIFVNWKELIMCNIKLLKALRVRKKMSGEKMPVK
MIGDILSAQLPHMQPYIRFCSRQLNGAALIQQKTDEAPDFKEFVKRLAMD
PRCRGMPLSSFILKPMQRVTRYPLIIKNILENTPENHPDHSHLKHALEKA
EELCSQVNEGVREKENSDRLEWIQAHVQCEGLSEQLVFNSVTNCLGPRKF
LHSGKLYKAKSNKELYGFLFNDFLLLTQITKPLGSSGTDKVFSPKSNLQY
KMYKTPIFLNEVLVKLPTDPSGDEPIFHISHIDRVYTLRAESINERTAWV
QKIKAASELYIETEKKKREKAYLVRSQRATGIGRLMVNVVEGIELKPCRS
HGKSNPYCEVTMGSQCHITKTIQDTLNPKWNSNCQFFIRDLEQEVLCITV
FERDQFSPDDFLGRTEIRVADIKKDQGSKGPVTKCLLLHEVPTGEIVVRLDLQLFDEP

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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 S315A mutation. 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

Nucleotide Sequence

ATGGCTCAGTTTCCAACACCTTTTGGTGGCAGCCTGGATATCTGGGCCATAACTGTAGAGGAAAGAGCGAAGCATGATCAGCAGTT
CCATAGTTTAAAGCCAATATCTGGATTCATTACTGGTGATCAAGCTAGAAACTTTTTTTTTCAATCTGGGTTACCTCAACCTGTTT
TAGCACAGATATGGGCACTAGCTGACATGAATAATGATGGAAGAATGGATCAAGTGGAGTTTTCCATAGCTATGAAACTTATCAAA
CTGAAGCTACAAGGATATCAGCTACCCTCTGCACTTCCCCCTGTCATGAAACAGCAACCAGTTGCTATTTCTAGCGCACCAGCATT
TGGTATGGGAGGTATCGCCAGCATGCCACCGCTTACAGCTGTTGCTCCAGTGCCAATGGGATCCATTCCAGTTGTTGGAATGTCTC
CAACCCTAGTATCTTCTGTTCCCACAGCAGCTGTGCCCCCCCTGGCTAACGGGGCTCCCCCTGTTATACAACCTCTGCCTGCATTT
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