

## ***MRC PPU REAGENTS***

### **Standard Operating Procedure**

#### **Preparation of ITSN1 S313A [1 – 1220]**

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

**Clone number:-** DU 27299

**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

# MRC PPU REAGENTS

## Clone Data Sheet

### ITSN1 S313A [1 - 1220]

Protein ITSN1 S313A [1 - 1220]

Clone number DU 27299

Species Human

Accession number NM\_001001132.1

Tags N-terminal GST

### Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL  
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL  
DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH  
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA  
WPLQGWQATFGGGDHPKSDLEVLFGGGLGSPNSRVEMAQFPTPFGGSLD  
IWAITVEERAKHDQQFHSLKPTISGFITGDQARNFFQSGLPQPVLAQIWA  
LADMNDGRMDQVEFSIAMKLIKLLKQGYQLPSALPPVMKQQPVAISSAP  
AFGMGGIASMPPLTAVAPVPMGSI PVVGMSP TLVSSVPTAAVPLANGAP  
PVIQPLPAFAHPAATLPKSSFSRSGPGSQLNTKLQKAQSFVAVSPPVA  
EWAVPQSSRLKYRQLFNSHDKTMSGHLTGPQARTILMQSSLPQAQLASIW  
NLSIDIDQDGKLTAEFFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRA  
GSGISVISSTSDVQRLPEEPVLEDEQQOLEKKLPVTFEDKKRENFERNL  
ELEKRRQALLEQQRKEQERLAQLERAQERKERERQEQERKRQLELEKQL  
EKQRELERQREERKEIEREAAKRELERQRLQLEWERNRQELLNQRNK  
EQEDIVVLKAKKKTLEFELEALNDKKHQLEGLQD IRCRLTTQRQEIEST  
NKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILNDQLKQVQQNSLHRD  
SLVTLKRALEAKELARQHLDQLEVEKETRSKLQEQIDIFNNQLKELREI  
HNKQQLQKQKSMEAERLKQKEQERKIIIELEKQKEEAQRRQERDKWLEH  
VQQEDEHQRPRLHEEEKLKREESVKKKDGEKQQAQDKLGRLFHQHQ  
EPAKPAVQAPWSTAEGKPLTISAQENVKVYRALLYPFESRSHDEITIQP  
GDIVMVKGEWVDESQTGEPGWLGGELKGTGWFPANYAEKIPENEVPAPV  
KPVTDSTAPAPKLALRETPAPLAVTSSEPSTTPNNWADFSSTWPTSTNE  
KPETDNWDAWAAQPSLTVPSAGQLRQSAFTPATATGSSPSPVLGQGEKV  
EGLQAQALYPWRAKKNHLNFKNDVITVLEQQDMWWFGEVQGGKQWFPK  
SYVKLISGPIRKSTSMDSGSSSESPASLKRVASPAKPVVSGEEFIAMTY  
ESSEQGD LTFQQGDVILVTKKDGWWTGTVDKAGVFPSPNYVRLKDLSEGS  
GTAGKTGSLGKKPEIAQVIASYTATGPEQLTLAPQLILIRKKNPGGWE  
GELQARGKKRQIGWFPANYVKLLSPGTSKITPTEPPKSTALAAVCQVIGM  
YDYTAQNDDLAFNKGQIINVLNKEDPDWWKGEVNGQVGLFSPNYVCLTT  
DMDPSQQWCSDLHLLDMLTPTERKRQGYIHELIVTEENYVNDLQLVTEIF  
QKPLMESELLTEKEVAMIFVNWELIMCNILKALRVRKMSGEKMPVK  
MIGDILSAQLPHMQPYIRFCSRQLNGAALIQQKTDEAPDFKEFVKRLAMD  
PRCRGMPLSSFILKPMQRVTRYPLIKNILENTPENHPDHSHLKHALEKA  
EELCSQVNEGVREKENSRLLEWIQAHVQCEGLSEQLVFNSVTNCLGPRKF  
LHSGKLYKAKSNKELYGFLFNDLLLITQITKPLGSSGTDKVFSPKSNLQY  
KMYKTFIFLNEVLVKLPTDPSGDEPIFHISHIDRVYTLRAESINERTAW  
QKKAASELYIETEKKKREKAYLVRSQRATGIGRLMVNVVEGIELKPCRS  
HGKSNPYCEVTMGSQCHITKTIQDTLNPKNWNSCQFFIRDLEQEVLCITV  
FERDQFSPDDFLGRTEIRVADIKKDQSGKGPVTKCLLLHEVPTGEIVVRLDLQLFDEP

## ***MRC PPU REAGENTS***

### **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 mutation. Residue S313 is equivalent to A550 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

## ***MRC PPU REAGENTS***

### **Nucleotide Sequence Of Insert**

ggatccccgaattcccgggtcgagATGGCTCAGTTTCCAACACCTTTTGGTGGCAGCCTGG  
ATATCTGGGCCATAACTGTAGAGGAAAGAGCGAAGCATGATCAGCAGTTCATAGTTTAAA  
GCCAATATCTGGATTCATTACTGGTGATCAAGCTAGAACTTTTTTTTTTCAATCTGGGTTA  
CCTCAACCTGTTTTAGCACAGATATGGGCACTAGCTGACATGAATAATGATGGAAGAATGG  
ATCAAGTGGAGTTTTTCCATAGCTATGAACTTATCAAACCTGAAGCTACAAGGATATCAGCT  
ACCCTCTGCACTTCCCCCTGTCATGAAACAGCAACCAGTTGCTATTTCTAGCGCACCAGCA  
TTTGGTATGGGAGGTATCGCCAGCATGCCACCGCTTACAGCTGTTGCTCCAGTGCCAATGG  
GATCCATTCCAGTTGTTGGAATGTCTCCAACCCTAGTATCTTCTGTTCCCACAGCAGCTGT  
GCCCCCCTGGCTAACGGGGCTCCCCCTGTTATACAACCTCTGCCTGCATTTGCTCATCCT  
GCAGCCACATTGCCAAAGAGTTCTTCCTTTAGTAGATCTGGTCCAGGGTCACAACCTAAACA  
CTAAATTACAAAAGGCACAGTCATTTGATGTGGCCAGTGTCCCACCAGTGGCAGAGTGGGC  
TGTTCCCTCAGTCATCAAGACTGAAATACAGGCAATTATTCAATAGTCATGACAAAACCTATG  
AGTGGACACTTAACAGGTCCCCAAGCAAGAACTATTCTTATGCAGTCAAGTTTACCACAGG  
CTCAGCTGGCTTCAATATGGAATCTTTCTGACATTGATCAAGATGGAAAACCTTACAGCAGA  
GGAATTTATCCTGGCAATGCACCTCATTTGATGTAGCTATGTCTGGCCAACCCTGCCACCT  
GTCCTGCCTCCAGAATACATTCCACCTTCTTTTAGAAGAGTTCGAGCTGGCAGTGGTATAT  
CTGTCATAAGCTCAACATCTGTAGATCAGAGGCTACCAGAGGAACCAGTTTTAGAAGATGA  
ACAACAACAATTAGAAAAGAAATTACCTGTAACGTTTGAAGATAAGAAGCGGGAGAACTTT  
GAACGTGGCAACCTGGAACCTGGAGAAAACGAAGGCAAGCTCTCCTGGAACAGCAGCGCAAGG  
AGCAGGAGCGCCTGGCCAGCTGGAGCGGGCGGAGCAGGAGAGGAAGGAGCGTGAGCGCCA  
GGAGCAAGAGCGCAAAAGACAACCTGGAACCTGGAGAAGCAACTGGAAAAGCAGCGGGAGCTA  
GAACGGCAGAGAGAGGAGGAGAGGAGGAAAGAAATTGAGAGGCGAGAGGCTGCAAAACGGG  
AACTTGAAAGGCAACGACAACCTTGAGTGGGAACGGAATCGAAGGCAAGAACTACTAAATCA  
AAGAAACAAAGAACAAGAGGACATAGTTGTACTGAAAGCAAAGAAAAAGACTTTGGAATTT  
GAATTAGAAGCTCTAAATGATAAAAAGCATCAACTAGAAGGGAAACTTCAAGATATCAGAT  
GTCGATTGACCACCCAAAGGCAAGAAATTGAGAGCACAAACAAATCTAGAGAGTTGAGAAT  
TGCCGAAATCACCCATCTACAGCAACAATTACAGGAATCTCAGCAAATGCTTGGAAAGACTT  
ATTCAGAAAAACAGATACTCAATGACCAATTAACAACAAGTTCAGCAGAACAGTTTGCACA  
GAGATTCACCTTGTACTACTTAAAAGAGCCTTAGAAGCAAAGAAGACTAGCTCGGCAGCACCT  
ACGAGACCAACTGGATGAAGTGGAGAAAAGAACTAGATCAAAACTACAGGAGATTGATATT  
TTCAATAATCAGCTGAAGGAACTAAGAGAAATACACAATAAGCAACAACCTCCAGAAGCAAA  
AGTCCATGGAGGCTGAACGACTGAAACAGAAAGAACAAGAACGAAAGATCATAGAATTAGA  
AAAACAAAAGAAGAAGCCCAAAGACGAGCTCAGGAAAGGGACAAGCAGTGGCTGGAGCAT  
GTGCAGCAGGAGGACGAGCATCAGAGACCAAGAAAACCTCACGAAGAGGAAAAACTGAAAA  
GGGAGGAGAGTGTCAAAAAGAAGGATGGCGAGGAAAAGGCAAACAGGAAGCACAAGACAA  
GCTGGGTGGCTTTTTCCATCAACACCAAGAACCAGCTAAGCCAGCTGTCCAGGCACCCTGG  
TCCACTGCAGAAAAGGTCCACTTACCATTTCTGCACAGGAAAATGTAAAAGTGGTGTATT  
ACCGGGCACTGTACCCCTTTGAATCCAGAAGCCATGATGAAATCACTATCCAGCCAGGAGA  
CATAGTCATGGTTAAAGGGGAATGGGTGGATGAAAGCCAAACTGGAGAACCCGGCTGGCTT  
GGAGGAGAATTAAGGAAAGACAGGGTGGTTCCCTGCAAACCTATGCAGAGAAAATCCCAG  
AAAATGAGGTTCCCGCTCCAGTGAACCAAGTACTGATTCAACATCTGCCCTGCCCCCAA  
ACTGGCCTTGCGTGAGACCCCCGCCCTTTGGCAGTAACCTCTTCAGAGCCCTCCACGACC  
CCTAATAACTGGGCCGACTTCAGCTCCACGTGGCCACCAGCACGAATGAGAAACCAGAAA  
CGGATAACTGGGATGCATGGGCAGCCCAGCCCTCTCTCACCGTTCCAAGTGCCGGCCAGTT  
AAGGCAGAGGTCCGCCTTTACTCCAGCCACGGCCACTGGCTCCTCCCCGTCTCCTGTGCTA  
GGCCAGGGTGAAAAGGTGGAGGGGCTACAAGCTCAAGCCCTATATCCTTGGAGAGCCAAAA  
AAGACAACCACTTAAATTTTAAACAAAATGATGTCATCACCGTCTTGAACAGCAAGACAT  
GTGGTGGTTTTGGAGAAGTTCAAGGTCAGAAGGGTTGGTTCCCCAAGTCTTACGTGAAACTC  
ATTTACAGGGCCCATAAGGAAGTCTACAAGCATGGATTCTGGTTCTTCAGAGAGTCTTGCTA

## ***MRC PPU REAGENTS***

GTCTAAAGCGAGTAGCCTCTCCAGCAGCCAAGCCGGTCGTTTCGGGAGAAGAATTTATTGC  
CATGTACACTTACGAGAGTTCTGAGCAAGGAGATTTAACCTTTCAGCAAGGGGATGTGATT  
TTGGTTACCAAGAAAGATGGTGACTGGTGGACAGGAACAGTGGGCGACAAGGCCGGAGTCT  
TCCCTTCTAACTATGTGAGGCTTAAAGATTCAGAGGGCTCTGGAAGTCTGGGAAAACAGG  
GAGTTTAGGAAAAAACCTGAAATTGCCAGGTTATTGCCTCATAACCCGCCACCGGCCCC  
GAGCAGCTCACTCTCGCCCCTGGTCAGCTGATTTTGTATCCGAAAAAGAACCAGGTGGAT  
GGTGGGAAGGAGAGCTGCAAGCACGTGGGAAAAAGCGCCAGATAGGCTGGTCCCAGCTAA  
TTATGTAAAGCTTCTAAGCCCTGGGACGAGCAAAATCACTCCAACAGAGCCACCTAAGTCA  
ACAGCATTAGCGGCAGTGTGCCAGGTGATTGGGATGTACGACTACACCGCGCAGAATGACG  
ATGAGCTGGCCTTCAACAAGGGCCAGATCATCAACGTCCTCAACAAGGAGGACCTGACTG  
GTGGAAAGGAGAAGTCAATGGACAAGTGGGGCTCTTCCCATCCAATTATGTGAAGCTGACC  
ACAGACATGGACCCAAGCCAGCAATGGTGTTCAGACTTACATCTCTTGGATATGTTGACCC  
CAACTGAAAGAAAGCGACAAGGATACATCCACGAGCTCATTGTCACCGAGGAGAACTATGT  
GAATGACCTGCAGCTGGTCACAGAGATTTTTCAAACCCCTGATGGAGTCTGAGCTGCTG  
ACAGAAAAAGAGGTTGCTATGATTTTTGTGAACTGGAAGGAGCTGATTATGTGTAATATCA  
AACTACTAAAAGCGCTGAGAGTCCGCAAGAAGATGTCCGGGGAGAAGATGCCTGTGAAGAT  
GATTGGAGACATCCTGAGCGCACAGCTGCCCGCATGTCAGCCCTACATCCGCTTCTGCAGC  
CGCCAGCTCAACGGGGCTGCCCTGATCCAGCAGAAGACGGACGAGGCCCCAGACTTCAAGG  
AGTTCGTCAAAAAGATTGGCAATGGATCCTCGGTGTAGAGGGATGCCACTCTCTAGTTTTAT  
ACTGAAGCCTATGCAACGGGTAACAAGATACCCACTGATCATTAAAAATATCCTGGAAAAC  
ACCCCTGAAAACCACCCGGACCACAGCCACTTGAAGCACGCCCTGGAGAAGGCGGAAGAGC  
TCTGTTCCAGGTGAACGAAGGGGTGCGGGAGAAGGAGAAGTCTGACCGGCTGGAGTGGAT  
CCAGGCCACGTGCAGTGTGAAGGCCTGTCTGAGCAACTTGTGTTCAATTCAGTGACCAAT  
TGCTTGGGGCCGCGCAAATTTCTGCACAGTGGGAAGCTCTACAAGGCCAAGAGCAACAAGG  
AGCTGTATGGCTTCCTTTTCAACGACTTCCTCCTGCTGACTCAGATCACGAAGCCTTTGGG  
GTCTTCTGGCACCGACAAAGTCTTCAGCCCCAAATCAAACCTGCAGTATAAAATGTATAAA  
ACACCTATTTTCTAAATGAGGTTCTAGTAAAATTACCCACCGACCTTCTGGAGACGAGC  
CCATCTTCCACATCTCCACATTGACCGCGTCTATACTCTCCGAGCAGAAAGCATAAAATGA  
AAGGATTGCCTGGGTGCAGAAAATCAAAGCTGCTTCTGAACTCTACATAGAGACTGAGAAA  
AAGAAGCGCGAGAAAGCGTACCTGGTCCGTTCCCAAAGGGCAACAGGCATTGGAAGGTTGA  
TGGTGAACGTGGTTGAAGGCATCGAGTTGAAACCCTGTCGGTACATGGAAAGAGCAACCC  
GTACTGTGAGGTGACCATGGGTTCCAGTGCCACATCACCAGACGATCCAGGACACTCTG  
AACCCCAAGTGAATTCCAACCTGCCAGTTCTTCATCCGAGACCTGGAGCAGGAAGTCCTCT  
GCATCACTGTGTTCGAGAGGGACCAGTTCTCACCAGATGATTTTTTGGGTCCGACGGAGAT  
CCGTGTGGCGGACATCAAGAAAGACCAGGGCTCCAAAGGTCCAGTTACGAAGTGTCTTCTG  
CTGCACGAAGTCCCACGGGAGAGATTGTGGTCCGCTTGGACCTGCAGTTGTTTGATGAGC  
CGtaggcggccgc