

## ***MRC PPU REAGENTS***

### **Standard Operating Procedure**

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

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

**Clone number:-** DU 28838

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 222, 816.93 daltons

Average Mass 222, 956.60 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 [1 - 1220]

Protein ITSN1 [1 - 1220]

Clone number DU 28838

Species Human

Accession number NM\_001001132.1

Tags N-terminal GST

Bacterially  
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL  
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL  
DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH  
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIA  
WPLQGWQATFGGGDHPFKSDLEVLFGGGLGSPNSRVEMAQFPPTFGGSLD  
IWAITVEERAKHDQQFHSLKPI SGFITGDOARNFFFQSGLPQPVLAQIWA  
LADMNNDGRMDQVEFSIAMKLIKLLKQGYQLPSALPPVMKQQPVAISSAP  
AFGMGGIASMPPLTAVAPVPMGSI PVVGMSPTLVSSVPTAAVPPLANGAP  
PVIQPLPAFAHPAATLPKSSSF SRSGPGSQLNTKLQKAQSFVDVASVPPVA  
EWAVPQSSRLKYRQLFN SHDKTMSGHLTGPOARTILMQSSLPQAQLASIW  
NLSDIDQDGKLTAEFFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRS  
GSGISVISSTSDQRLPEEPVLEDEQQOLEKKLPVTFEDKKRENFERGNL  
ELEKRRQALLEQQRKEQERLAQLERAQERKERERQEQERKRQLELEKQL  
EKQRELERQREEERKEIERREAAKRELERQRLWERNRRQELLNQRNK  
EQEDIVVLKAKKKTLEFELEALNDKHKHGLEKQLDIRCRLTTQRQEIEST  
NKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILNDQLKQVQQNSLHRD  
SLVTLKRALEAKELARQHRLDQLEVEKETRSKLQEQIDIFNNQLKELREI  
HNKQQLQKQKSMEAEERLKQKEQERKIELEKQKEEAQRAQERDKQWLEH  
VQQEDEHQRPKRLHEEEKLKREESVKKKDGEKQEAQDKLGRLFHQHQ  
EPAKPAVQAPWSTA EKGPLTISAQENVKVYRYRALYPFESRSHDEITIQP  
GDIVMVG EWVDESQTGEPGWLGGELKGTGWFPANYAEKIPENEVPAPV  
KPVTDST SAPAPKLALRET PAVLTSSEPSTTPNNWADFSSTWPTSTNE  
KPETDNDAWAAQPSLTVPSAGQLRQSAFTPATATGSSPSPVLGQGEKV  
EGLQAQALYPWRAKDNHLNFNKNDVITVLEQQDMWWFGEVQGGKGFPPK  
SYVKLISGPIRKSTSMDSGSSPASPILKRVASPAKPVVSGEEFIAMTTY  
ESSEQGDLTFFQQGDVILVTKKGDWWTGTVDKAGVFP SNYVRLKDSEGS  
GTAGKTGSLGKKPEIAQVIASYTATGPEQLTLAPGQLILIRKKNPGGWEE  
GELQARGKKRQIGWFPANYVKLLSPGTSKITPTEPPKSTALAAVCQVIGM  
YDYTAQNDELAFNKGQI INVLNKEDPDWWKGEVNGQVGLFSPSNYVKLTT  
DMDPSQQWCSDLHLLDMLTPTERKRQGYIHELIVTEENYVNDLQLVTEIF  
QKPLMESELLTEKEVAMIFVNWKEIMCNIKLLKALRVRKKMSGEKMPVK  
MIGDILSAQLPHMQPYIRFC SRQLNGAALIQOKTDEAPDFKFEVFKRLAMD  
PRCRGMPLSSFILKPMQRVTRYPLI IKNILENTPENHPDHSHLKHLEKA  
EELCSQVNEGVREKENS DRLEWIAHVQCEGLSEQLVFN SVTNCGLGRKF  
LHSGKLYKAKSNKELYGFLFND FLLLTQITKPLGSSGTDKVFSPKSNLOY  
KMYKTPIFLNEVLVKLPTDPSGDEPIFHISHIDRVYTLRAESINERTAWV  
QKIKAASELYIETEKKKREKAYLVRSQRATGIGRLMVNVVEGIELKPCRS  
HGKSNPYCEVTMG SQCHITKTIQDTLNPKNWNSNCQFFIRDLEQEVLCITV  
FERDQFSPDDFLGRTEIRVADIKKDGSKGPVTKCLLLHEVPTGEIVVRLDLQLFDEP

## ***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.
- 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**

ctcagagATGGCTCAGTTTCCAACACCTTTTGGTGGCAGCCTGGATATCTGGGCCATAACTG  
TAGAGGAAAGAGCGAAGCATGATCAGCAGTTCCATAGTTTAAAGCCAATATCTGGATTCAT  
TACTGGTGATCAAGCTAGAACTTTTTTTTTTCAATCTGGGTACCTCAACCTGTTTTAGCA  
CAGATATGGGCACTAGCTGACATGAATAATGATGGAAGAATGGATCAAGTGGAGTTTTCCA  
TAGCTATGAACTTATCAAACCTGAAGCTACAAGGATATCAGCTACCCTCTGCACCTCCCCC  
TGTCATGAAACAGCAACCAGTTGCTATTTCTAGCGCACCAGCATTTGGTATGGGAGGTATC  
GCCAGCATGCCACCGCTTACAGCTGTTGCTCCAGTGCCAATGGGATCCATTCCAGTTGTTG  
GAATGTCTCCAACCCTAGTATCTTCTGTTCCCACAGCAGCTGTGCCCCCCTGGCTAACGG  
GGCTCCCCCTGTTATAACAACCTCTGCCTGCATTTGCTCATCCTGCAGCCACATTGCCAAG  
AGTTCTTCCTTTAGTAGATCTGGTCCAGGGTCACAACTAAACACTAAATTACAAAAGGCAC  
AGTCATTTGATGTGGCCAGTGTCCCACCAGTGGCAGAGTGGGCTGTTCCCTCAGTCATCAAG  
ACTGAAATACAGGCAATTATTCAATAGTCATGACAAAACCTATGAGTGGACACTTAACAGGT  
CCCCAAGCAAGAACTATTCTTATGCAGTCAAGTTTACCACAGGCTCAGCTGGCTTCAATAT  
GGAATCTTTCTGACATTGATCAAGATGGAAAACCTTACAGCAGAGGAATTTATCCTGGCAAT  
GCACCTCATTGATGTAGCTATGTCTGGCCAACCCTGCCACCTGTCTGCCTCCAGAATAC  
ATTCACCTTCTTTTAGAAGAGTTCGATCTGGCAGTGGTATATCTGTGCATAAGCTCAACAT  
CTGTAGATCAGAGGCTACCAGAGGAACCAGTTTLAGAAGATGAACAACAACAATTAGAAAA  
GAAATTACCTGTAACGTTTGAAGATAAGAAGCGGGAGAACTTTGAACGTGGCAACCTGGAA  
CTGGAGAAACGAAGGCAAGCTCTCCTGGAACAGCAGCGCAAGGAGCAGGAGCGCCTGGCCC  
AGCTGGAGCGGGCGGAGCAGGAGAGGAAGGAGCGTGAGCGCCAGGAGCAAGAGCGCAAAAAG  
ACAACCTGGAACCTGGAGAAGCAACTGGAAAAGCAGCGGGAGCTAGAACGGCAGAGAGAGGAG  
GAGAGGAGGAAAGAAATTGAGAGGCGAGAGGCTGCAAAAACGGGAACCTTGAAGGCAACGCAC  
AACTTGAGTGGGAACGGAATCGAAGGCAAGAACTACTAAATCAAAGAAACAAAGAACAAGA  
GGACATAGTTGTACTGAAAGCAAAGAAAAAGACTTTGGAATTTGAATTAGAAGCTCTAAAT  
GATAAAAAGCATCAACTAGAAAGGGAAACTTCAAGATATCAGATGTCGATTGACCACCCAAA  
GGCAAGAAATTGAGAGCACAAAACAATCTAGAGAGTTGAGAATTGCCGAAATCACCCATCT  
ACAGCAACAATTACAGGAATCTCAGCAAATGCTTGGAAAGACTTATTCAGAAAAACAGATA  
CTCAATGACCAATTAACAACAAGTTTCAAGCAGAACAGTTTGCACAGAGATTCACCTTGTTACAC  
TTAAAAGAGCCTTAGAAGCAAAGAAGTACTCGGCAGCACCTACGAGACCAACTGGATGA  
AGTGGAGAAAGAACTAGATCAAAACTACAGGAGATTGATATTTTCAATAATCAGCTGAAG  
GAACTAAGAGAAATACACAATAAGCAACAACCTCCAGAAGCAAAAAGTCCATGGAGGCTGAAC  
GACTGAAACAGAAAGAACAAGAACGAAAGATCATAGAATTAGAAAAACAAAAAGAAGAAGC  
CCAAAGACGAGCTCAGGAAAGGGACAAGCAGTGGCTGGAGCATGTGCAGCAGGAGGACGAG  
CATCAGAGACCAAGAAAACCTCCACGAAGAGGAAAAACTGAAAAGGGAGGAGAGTGTCAAAA  
AGAAGGATGGCGAGGAAAAAGGCAAACAGGAAGCACAAGACAAGCTGGGTTCGGCTTTTCCA  
TCAACACCAAGAACCAGCTAAGCCAGCTGTCCAGGCACCCTGGTCCACTGCAGAAAAAGGT  
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TTGAATCCAGAAGCCATGATGAAATCACTATCCAGCCAGGAGACATAGTCATGGTTAAAGG  
GGAATGGGTGGATGAAAGCCAAACTGGAGAACCCTGGCTGGCTTGGAGGAGAATTAAGAAGGA  
AAGACAGGGTGGTTCCCTGCAAACCTATGCAGAGAAAAATCCAGAAAATGAGGTTCCCGCTC  
CAGTGAACCCAGTGACTGATTCAACATCTGCCCTGCCCCAAACTGGCCTTGCCTGAGAC  
CCCCGCCCTTTGGCAGTAACCTCTTCCAGAGCCCTCCACGACCCTAATAACTGGGCCGAC  
TTCAGCTCCACGTGGCCACCAGCACGAATGAGAAACCAGAAACGGATAACTGGGATGCAT  
GGGCAGCCCAGCCCTCTCTCACCGTTCCAAGTGCCGGCCAGTTAAGGCAGAGGTCCGCCTT  
TACTCCAGCCACGGCCACTGGCTCCTCCCCGTCTCCTGTGCTAGGCCAGGGTGAAGAAGGTG  
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TTAACAAAAATGATGTCATCACCGTCTGGAACAGCAAGACATGTGGTGGTTTGGAGAAGT  
TCAAGGTCAGAAGGGTTGGTTCCCAAGTCTTACGTGAAACTCATTTCCAGGGCCATAAGG  
AAGTCTACAAGCATGGATTCTGGTTCTTCCAGAGAGTCTGCTAGTCTAAAGCGAGTAGCCT

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CTCCAGCAGCCAAGCCGGTTCGTTTCGGGAGAAGAATTTATTGCCATGTACACTTACGAGAG  
TTCTGAGCAAGGAGATTTAACCTTTTCAGCAAGGGGATGTGATTTTGGTTACCAAGAAAGAT  
GGTGACTGGTGGACAGGAACAGTGGGCGACAAGGCCGGAGTCTTCCCTTCTAACTATGTGA  
GGCTTAAAGATTCAGAGGGCTCTGGAAGTCTGGGAAAACAGGGAGTTTAGGAAAAAAACC  
TGAAATTGCCAGGTTATTGCCTCATACACCGCCACCGGCCCCGAGCAGCTCACTCTCGCC  
CCTGGTCAGCTGATTTTGTATCCGAAAAAGAACCCAGGTGGATGGTGGGAAGGAGAGCTGC  
AAGCACGTGGGAAAAAGCGCCAGATAGGCTGGTTCCCAGCTAATTATGTAAAGCTTCTAAG  
CCCTGGGACGAGCAAAATCACTCCAACAGAGCCACCTAAGTCAACAGCATTAGCGGCAGTG  
TGCCAGGTGATTGGGATGTACGACTACACCGCGCAGAATGACGATGAGCTGGCCTTCAACA  
AGGGCCAGATCATCAACGTCCTCAACAAGGAGGACCCCTGACTGGTGGAAAGGAGAAGTCAA  
TGGACAAGTGGGGCTCTTCCCATCCAATTATGTGAAGCTGACCACAGACATGGACCCAAGC  
CAGCAATGGTGTTCAGACTTACATCTCTTGGATATGTTGACCCCAACTGAAAAGAAAGCGAC  
AAGGATACATCCACGAGCTCATTGTCACCGAGGAGAAGTATGTGAATGACCTGCAGCTGGT  
CACAGAGATTTTTCAAAAACCCCTGATGGAGTCTGAGCTGCTGACAGAAAAAGAGGTTGCT  
ATGATTTTTGTGAAGTGGAAAGGAGCTGATTATGTGTAATATCAAATACTAAAAGCGCTGA  
GAGTCCGCAAGAAGATGTCCGGGGAGAAGATGCCTGTGAAGATGATTGGAGACATCCTGAG  
CGCACAGCTGCCGCACATGCAGCCCTACATCCGCTTCTGCAGCCGCCAGCTCAACGGGGCT  
GCCCTGATCCAGCAGAAGACGGACGAGGCCCCAGACTTCAAGGAGTTCGTCAAAAGATTGG  
CAATGGATCCTCGGTGTAGAGGGATGCCACTCTCTAGTTTTATACTGAAGCCTATGCAACG  
GGTAACAAGATACCCACTGATCATTAAAAATATCCTGGAAAACACCCCTGAAAACCACCCG  
GACCACAGCCACTTGAAGCACGCCCTGGAGAAGGCGGAAGAGCTCTGTTCCCAGGTGAACG  
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TCAACGACTTCCTCCTGCTGACTCAGATCACGAAGCCTTTGGGGTCTTCTGGCACCGACAA  
AGTCTTCAGCCCCAAATCAAACCTGCAGTATAAAATGTATAAAACACCTATTTTCCTAAAT  
GAGGTTCTAGTAAAATTACCCACCGACCCTTCTGGAGACGAGCCCATCTTCCACATCTCCC  
ACATTGACCGCTCTATACTCTCCGAGCAGAAAGCATAAATGAAAGGACTGCCTGGGTGCA  
GAAAATCAAAGCTGCTTCTGAACTCTACATAGAGACTGAGAAAAAGAAGCGCGAGAAAGCG  
TACCTGGTCCGTTCCCAAAGGGCAACAGGCATTGGAAGGTTGATGGTGAACGTGGTTGAAG  
GCATCGAGTTGAAACCCGTGTCGGTACATGGAAAGAGCAACCCGTACTGTGAGGTGACCAT  
GGGTTCCCAGTGCCACATCACCAAGACGATCCAGGACACTCTGAACCCCAAGTGGAAATTC  
AACTGCCAGTTCTTCATCCGAGACCTGGAGCAGGAAGTCTCTGCATCACTGTGTTTCGAGA  
GGGACCAGTTCTCACCAGATGATTTTTTGGGTCCGACGGAGATCCGTGTGGCGGACATCAA  
GAAAGACCAGGGCTCCAAAGGTCCAGTTACGAAGTGTCTTCTGCTGCACGAAGTCCCCACG  
GGAGAGATTGTGGTCCGCTTGACCTGCAGTTGTTTGTATGAGCCGtaggcggccgc