

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of CTNNA1 [1 – 906]**

**Enzyme description:-** CTNNA1 [1 - 906]

**Clone number:-** DU 25458

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 127, 188.32 daltons

Average Mass 127, 268.84 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.84

**Purity:-** >80 %

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 270 mM 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

#### CTNNA1 [1 - 906]

|                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Protein</u>                       | CTNNA1 [1 - 906]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <u>Clone number</u>                  | DU 25458                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <u>Species</u>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <u>Accession number</u>              | P35221                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <u>Tags</u>                          | N-terminal GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <u>Bacterially expressed protein</u> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br/>LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA<br/>VLDIRYGVSRAYSCKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH<br/>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS<br/>KYIAWPLQGWQATFGGGDHPPKSDLEVLFFQGPLGSPEF<b>MTAVHAGNINF</b><br/><b>KWDPKSLEIRTLAVERLLEPLVTQVTTLVNTNSKGPSNKKRGRSKKAHV</b><br/><b>LAASVEQATENFLEKGDKIAKESQFLKEELVAAVEDVRKQGDLMKAAAG</b><br/><b>EFADDPCCSSVKRGNMVRARALLSAVTRLLILADMADVYKLLVQLKVVE</b><br/><b>DGILKLRNAGNEQDLGIQYKALKPEVDKLNIMAARKQELKDVGHRDQM</b><br/><b>AAARGILQKNVPILYTASQACLQHPDVAAYKANRDLIYKQLQQAVTGIS</b><br/><b>NAAQATASDDASQHQQGGGGELAYALNNFDKQIIVDPLSFSEERFRPSL</b><br/><b>EERLESIISGAALMADSSCTRDDRERIVAECNAVRQALQDLLSEYMGN</b><br/><b>AGRKERSDALNSAIDKMTKKTRDLRRQLRKAVMDHVSDSFLETNPVLLV</b><br/><b>LIEAAKNGNEKEVKEYAQVFREHANKLIEVANLACSI SNNEEGVKLVRM</b><br/><b>SASQLEALCPQVINAALALAAKPQSKLAQENMDLFKEQWEKQVRVLTDA</b><br/><b>VDDITSIDDFLAVSENHILEDVNKCVIALQEKDVDGLDRTAGAIRGRAA</b><br/><b>RVIHVVTSEMDNYEPGVYTEKVLEATKLLSNTVMPRFTEQVEAAVEALS</b><br/><b>SDPAQPMDENEFIDASRLVYDGI RDIRKAVLMIRTPEELDDSDFETEDF</b><br/><b>DVRSRTSVQTEDDQLIAGQSARAQMAQLPQEQAKIAEQVASFQEEKSK</b><br/><b>LDAEVSKWDDSGNDIIVLAKQMCMMEMTDFTRGKGPLKNTSDVISAA</b><br/><b>KKIAEAGSRMDKLGRTIADHCPDSACKQDLLAYLQRIALYCHQLNICKS</b><br/><b>VKAEVQNLGGELVVSQVDSAMSLIQAAKNLMNAVQTVKASYVASTKYQ</b><br/><b>KSQGMASLNLPAVSWKMKAPKKPLVKREKQDETQTKIKRASQKKHVNP</b><br/><b>VQALSEFKAMDSI</b></p> |
| <u>Native sequence</u>               | <p>Amino acids M1 – I906 (end) of human CTNNA1.<br/>Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

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Protease cleavage      PreScission (LEVLFQGP) residues 221 - 228

Cloning sites            *Eco*R1 and *Not*I sites of pGEX6P-1

### Nucleotide Sequence Of Insert

gaattcATGACTGCTGTCCATGCAGGCAACATAAACTTCAAGTGGGATCCTAAAAGTCTAGAGATC  
AGGACTCTGGCAGTTGAGAGACTGTTGGAGCCTCTTGTTACACAGGTTACAACCCTTGTAACACC  
AATAGTAAAGGGCCCTCTAATAAGAAGAGAGGTCGTTCTAAGAAGGCCCATGTTTTGGCTGCATCT  
GTTGAACAAGCAACTGAGAATTTCTTGGAGAAGGGGGATAAAATTGCGAAGGAGAGCCAGTTTCTC  
AAGGAGGAGCTTGTGGCTGCTGTAGAAGATGTTGAAAACAAGGTGATTTGATGAAGGCTGCTGCA  
GGAGAGTTCGCAGATGATCCCTGCTCTTCTGTGAAGCGAGGCAACATGGTTTCGGGCAGCTCGAGCT  
TTGCTCTCTGCTGTTACCCGGTTGCTGATTTTGGCTGACATGGCAGATGTCTACAAATTACTTGTT  
CAGCTGAAAGTTGTGGAAGATGGTATCTTGAAGTTGAGGAATGCTGGCAATGAACAAGACTTAGGA  
ATCCAGTATAAAGCCCTAAAACCTGAAGTGGATAAGCTGAACATTATGGCAGCCAAAAGACAACAG  
GAATTGAAAGATGTTGGCCATCGTGATCAGATGGCTGCAGCTAGAGGAATCCTGCAGAAGAACGTT  
CCGATCCTCTATACTGCATCCCAGGCATGCCTACAGCACCCCTGATGTCGCAGCCTATAAGGCCAAC  
AGGGACCTGATATACAAGCAGCTGCAGCAGGCGGTACAGGCATTTCCAATGCAGCCCAGGCCACT  
GCCTCAGACGATGCCTCACAGCACCCAGGGTGGAGGAGGAGGAGAAGTGGCATATGCACTCAATAAC  
TTTGACAAACAAATCATTTGTGGACCCCTTGAGCTTCAGCGAGGAGCGCTTTAGGCCTTCCCTGGAG  
GAGCGTCTGGAAGCATCATTAGTGGGGCTGCCTTGATGGCCGACTCGTCCTGCACGCGTGATGAC  
CGTCGTGAGCGAATTGTGGCAGAGTGTAATGCTGTCCGCCAGGCCCTGCAGGACCTGCTTTCGGAG  
TACATGGGCAATGCTGGACGTAAAGAAAGAAGTGATGCACTCAATTCTGCAATAGATAAAAATGACC  
AAGAAGACCAGGGACTTGCGTAGACAGCTCCGCAAAGCTGTCATGGACCACGTTTCAGATTCTTTC  
CTGGAAACCAATGTTCCACTTTTGGTATTGATTGAAGCTGCAAAGAATGGAAATGAGAAAGAAGTT  
AAGGAGTATGCCCAAGTTTTCCGTGAACATGCCAACAAATTGATTGAGGTTGCCAACTTGCCCTGT  
TCCATCTCAAATAATGAAGAAGGTGTAAAGCTTGTTTGAATGTCTGCAAGCCAGTTAGAAGCCCTC  
TGTCCTCAGGTTATTAATGCTGCACTGGCTTTAGCAGCAAAACCACAGAGTAACTGGCCCAAGAG  
AACATGGATCTTTTTTAAAGAACAATGGGAAAAACAAGTCCGTGTTCTCACAGATGCTGTGATGAC  
ATTACTTCCATTGATGACTTCTTGGCTGTCTCAGAGAATCACATTTTGGAAAGATGTGAACAAATGT  
GTCATTGCTCTCCAAGAGAAGGATGTGGATGGCCTGGACCGCACAGCTGGTGCAATTCGAGGCCGG  
GCAGCCCGGGTCATTCACGTAGTCACCTCAGAGATGGACAACATATGAGCCAGGAGTCTACACAGAG  
AAGGTTCTGGAAGCCACTAAGCTGCTCTCCAACACAGTCATGCCACGTTTTTACTGAGCAAGTAGAA  
GCAGCCGTGGAAGCCCTCAGCTCGGACCCTGCCCAGCCCATGGATGAGAATGAGTTTATCGATGCT  
TCCCGCCTGGTATATGATGGCATCCGGGACATCAGGAAAGCAGTGCTGATGATAAGGACCCCTGAG  
GAGTTGGATGACTCTGACTTTGAGACAGAAGATTTTGATGTCAGAAGCAGGACGAGCGTCCAGACA  
GAAGACGATCAGCTGATAGCTGGCCAGAGTGCCCGGGCGATCATGGCTCAGCTTCCCCAGGAGCAA  
AAAGCGAAGATTGCGGAACAGGTGGCCAGCTTCCAGGAAGAAAAGAGCAAGCTGGATGCTGAAGTG  
TCCAAATGGGACGACAGTGGCAATGACATCATTTGTGCTGGCCAAGCAGATGTGCATGATTATGATG  
GAGATGACAGACTTTACCCGAGGTAAAGGACCACTCAAAAATACATCGGATGTCATCAGTGCTGCC  
AAGAAAATTGCTGAGGCAGGATCCAGGATGGACAAGCTTGGCCGCACCATTGCAGACCATTGCCCC  
GACTCGGCTTGCAAGCAGGACCTGCTGGCCTACCTGCAACGCATCGCCCTCTACTGCCACCAGCTG  
AACATCTGCAGCAAGGTCAAGGCCGAGGTGCAGAATCTCGGCGGGGAGCTTGTTGTCTCTGGGGTG  
GACAGCGCCATGTCCCTGATCCAGGCAGCCAAGAAGCTTGATGAATGCTGTGGTGCAGACAGTGAAG  
GCATCCTACGTCGCTCTACCAAATACCAAAAGTCACAGGGTATGGCTTCCCTCAACCTTCTGCT  
GTGTCATGGAAGATGAAGGCACCAGAGAAAAAGCCATTGGTGAAGAGAGAGAAACAGGATGAGACA  
CAGACCAAGATTAAACGGGCATCTCAGAAGAAGCACGTGAACCCGGTGCAGGCCCTCAGCGAGTTC  
AAAGCTATGGACAGCATCtaagcgccg

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