*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of CTRC3 [1 – 619]

**Enzyme description:-** CRTC3 [1 - 619]

**Clone number:-** DU 3622

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method**:- GSH Sepharose

**Calculated molecular mass:-**
Monoisotopic 94, 681.40 daltons
Average Mass 94, 740.61 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 6.28

**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

## CRTC3 [1 - 619]

| | |
|---|---|
| **Protein** | CRTC3 [1 - 619] |
| **Clone number** | DU 3622 |
| **Species** | Human |
| **Accession number** | AY360173 |
| **Tags** | N-terminal GST |
| **Bacterially expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br>LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA<br>VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH<br>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS<br>KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGYPRIRPCGT**MAASPG**<br>**SGSANPRKFSEKIALHTQRQAEETRAFEQLMTDLTLSRVQFQKLQQLRL**<br>**TQYHGGSLPNVSQLRSSASEFQPSFHQADNVRGTRHHGLVERPSRNRFH**<br>**PLHRRSGDKPGRQFDGSAFGANYSSQPLDESWPRQQPPWKDEKHPGFRL**<br>**TSALNRTNSDSALHTSALSTKPQDPYGGGGQSAWPAPYMGFCDGENNGH**<br>**GEVASFPGPLKEENLLNVPKPLPKQLWETKEIQSLSGRPRSCDVGGGNA**<br>**FPHNGQNLGLSPFLGTLNTGGSLPDLTNLHYSTPLPASLDTTDHHFGSM**<br>**SVGNSVNNIPAAMTHLGIRSSSGLQSSRSNPSIQATLNKTVLSSSLNNH**<br>**PQTSVPNASALHPSLRLFSLSNPSLSTTNLSGPSRRRQPPVSPLTLSPG**<br>**PEAHQGFSRQLSSTSPLAPYPTSQMVSSDRSQLSFLPTEAQAQVSPPPP**<br>**YPAPQELTQPLLQQPRAPEAPAQQPQAASSLPQSDFQLLPAQGSSLTNF**<br>**FPDVGFDQQSMRPGPAFPQQVPLVQQGSRELQDSFHLRPSPYSNCGSLP**<br>**NTILPEDSSTSLFKDLNSALAGLPEVSLNVDTPFPLEEELQIEPLSLDG**<br>**LNMLSDSSMGLLDPSVEETFRADRL** |
| **Native sequence** | Amino acids M1 – L619 (end) of human CTRC3.<br>Residue M240 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** | *Eco*R1 sites of pGEX6P-3 |

**Nucleotide Sequence of insert**

attcgcccttgcggtaccATGGCCGCCTCGCCGGGCTCGGGCAGCGCCA
ACCCGCGGAAGTTCAGTGAGAAGATCGCGCTGCACACGCAGAGACAGGC
CGAGGAGACGCGGGCCTTCGAGCAGCTCATGACCGACCTCACCCTGTCG
CGGGTTCAATTTCAGAAGCTTCAGCAACTGCGCCTTACACAGTACCATG
GAGGATCCTTACCAAATGTGAGCCAGCTGCGGAGCAGTGCGTCAGAGTT
TCAGCCGTCATTTCACCAAGCTGATAATGTTCGGGGAACCCGCCATCAC
GGGCTGGTGGAGAGGCCATCCAGGAACCGCTTCCACCCCCTCCACCGAA
GGTCTGGGGACAAGCCAGGGCGACAATTTGATGGTAGTGCTTTTGGAGC
CAATTATTCCTCACAGCCTCTGGATGAGAGTTGGCCAAGGCAGCAGCCT
CCTTGGAAAGACGAAAAGCATCCTGGGTTCAGGCTGACATCTGCACTTA
ACAGGACCAATTCTGATTCTGCTCTTCACACGAGTGCTCTGAGTACCAA
GCCCCAGGACCCCTATGGAGGAGGGGGCCAGTCGGCCTGGCCTGCCCCA
TACATGGGTTTCTGTGATGGTGAGAATAATGGACATGGGGAAGTAGCAT
CTTTCCCTGGCCCATTGAAAGAAGAGAATCTGTTAAATGTTCCGAAGCC
ACTGCCAAAACAACTGTGGGAGACCAAGGAGATTCAGTCCCTGTCAGGA
CGCCCTCGATCCTGTGATGTTGGAGGTGGCAATGCTTTTCCACATAATG
GTCAAAACCTAGGCCTCTCACCCTTCTTGGGGACTTTGAACACTGGAGG
GTCATTGCCAGATCTAACCAACCTCCACTACTCGACACCCCTGCCAGCC
TCCCTGGACACCACCGACCACCACTTTGGCAGTATGAGTGTGGGGAATA
GTGTGAACAACATCCCAGCTGCTATGACCCACCTGGGTATAAGAAGCTC
CTCTGGTCTCCAGAGTTCTCGGAGTAACCCCTCCATCCAAGCCACGCTC
AATAAGACTGTGCTCTCCTCTTCCTTAAATAACCACCCACAGACATCTG
TTCCCAACGCATCTGCTCTTCACCCTTCGCTCCGTCTGTTTTCCCTTAG
CAACCCATCTCTTTCCACCACAAACCTGAGCGGCCCGTCTCGGCGTCGG
CAGCCTCCCGTCAGCCCTCTCACGCTTTCTCCTGGCCCTGAAGCACATC
AAGGTTTCAGCAGACAGCTGTCTTCAACCAGCCCACTGGCCCCATATCC
TACCTCCCAGATGGTGTCCTCAGACCGAAGCCAACTTTCCTTTCTGCCC
ACAGAAGCTCAAGCCCAGGTGTCGCCGCCACCCCCTTACCCTGCACCCC
AGGAGCTCACCCAGCCCCTCCTGCAGCAGCCCCGCGCCCCTGAGGCCCC
TGCCCAGCAGCCCCAGGCAGCCTCCTCACTGCCACAGTCAGACTTTCAG
CTTCTCCCGGCCCAGGGCTCATCTTTGACCAACTTCTTCCCAGATGTGG
GTTTTGACCAGCAGTCCATGAGGCCAGGCCCTGCCTTTCCTCAACAGGT
GCCTCTGGTGCAACAAGGTTCCCGAGAACTGCAGGACTCTTTTCATTTG
AGACCAAGCCCGTATTCCAACTGCGGGAGTCTCCCGAACACCATCCTGC
CAGAAGACTCCAGCACCAGCCTGTTCAAAGACCTCAACAGTGCGCTGGC
AGGCCTGCCTGAGGTCAGCCTGAACGTGGACACTCCATTTCCACTGGAA
GAGGAGCTGCAGATTGAACCCCTGAGCCTGGACGGACTCAACATGTTAA
GTGACTCCAGCATGGGCCTGCTGGACCCCTCTGTTGAAGAGACGTTTCG
AGCTGACAGACTGtgaggtaccgcaagggcgaattc