

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-CHIP 2-303

<u>Enzyme description:-</u>	CHIP 2-303
<u>Clone number:-</u>	DU1993
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	15mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	61509
Average Mass	61547
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.25
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-80°C

Assay:-

Ubiquitin chain assay with UBE1, Ubiquitin, UBE2D1. Adding CHIP will increase the amount of long isopeptide chains as monitored by immunoblotting against Ubiquitin.

Assay buffer: 25mM HEPES pH 7.5, 1mM DTT, 10mM Mg-acetate, 0.2mM ATP.

Assay conditions: 2h at 30°C – 37°C.

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Clone Data Sheet

Protein name GST-CHIP 2-303

<u>Protein</u>	CHIP 2-303
<u>Synonyms</u>	STIP1 homology and U-Box containing protein 1; serologically defined colon cancer antigen 7; carboxy terminus of Hsp70p-interacting protein; heat shock protein A binding protein 2 (c-terminal) (CHIP)
<u>Clone Number</u>	DU1993
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q9UNE7
<u>Tags</u>	N-terminal GST
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSD <u>LEVLFQGPLGSKGKEEKEGGARLGAGGGSP</u> EKSPSAQELKEQGNRLFVGRKYPEAAACYGRAITRNPLVAVYYTNRALCYLKMQQHEQALADCRRALELDGQSVKAHFFLGQCQLEMESYDEAIANLQRAYSLAKEQRLNFGDDIPALRIAKKRWNSIEERRIHQESLHLSRLIAAERERELEECQRNHEGDEDDSHVRAQQACIEAKHDKYMADMDELFSQVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Native sequence	2-303 in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamH1 / Not1

**DNA sequence of
insert**

GGATCCAAGGGCAAGGAGGAGAAGGAGGGCGGCGCACGGCTGGGCGCTGG
CGGCGGAAGCCCCGAGAAGAGCCCAGCGCGCAGGAGCTCAAGGAGCAGG
GCAATCGTCTGTTCGTGGGCCGAAAGTACCCGGAGGCGGCGGCCTGCTAC
GGCCGCGGATCACCCGGAACCCGCTGGTGGCCGTGTATTACACCAACCG
GGCCTTGTGCTACCTGAAGATGCAGCAGCACGAGCAGGCCCTGGCCGACT
GCCGGCGCGCCCTGGAGCTGGACGGGCAGTCTGTGAAGGCGCACTTCTTC
CTGGGGCAGTGCCAGCTGGAGATGGAGAGCTATGATGAGGCCATCGCCAA
TCTGCAGCGAGCTTACAGCCTGGCCAAGGAGCAGCGGCTGAACTTCGGGG
ACGACATCCCAGCGCTCTTCGAATCGCGAAGAAGAAGCGCTGGAACAGC
ATTGAGGAGCGGCGCATCCACCAGGAGAGCGAGCTGCACTCCTACCTCTC
CAGGCTCATTGCCGCGGAGCGTGAGAGGGAGCTGGAAGAGTGCCAGCGAA
ACCACGAGGGTGATGAGGACGACAGCCACGTCCGGGCCCAGCAGGCCTGC
ATTGAGGCCAAGCACGACAAGTACATGGCGGACATGGACGAGCTTTTTTC
TCAGGTGGATGAGAAGAGGAAGAAGCGAGACATCCCCGACTACCTGTGTG
GCAAGATCAGCTTTGAGCTGATGCGGGAGCCGTGCATCACGCCAGTGGC
ATCACCTACGACCGCAAGGACATCGAGGAGCACCTGCAGCGTGTGGGTCA
TTTTGACCCCGTGACCCGAGCCCCCTGACCCAGGAACAGCTCATCCCCA
ACTTGGCTATGAAGGAGGTTATTGACGCATTCATCTCTGAGAAATGGCTGG
GTGGAGGACTACTGAGCGGCCGC