

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CHIP [2 - 303]

Enzyme description:- CHIP [2 – 303]

Clone number:- DU 1993

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 61, 509.84 daltons
Average Mass 61, 549.18 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.66

Purity:- 85 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Ubiquitin assay

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Clone Data Sheet

CHIP [2 - 303]

<u>Protein</u>	CHIP [2 - 303]
<u>Clone Number</u>	DU 1993
<u>Species</u>	Human
<u>Accession number</u>	NM_005852
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGPLGSKGKEEKEGGARLGA GGGSPEKSPSAQELKEQGNRLFVGRKYPEAAACYGRAITRNPLVAVYYT NRALCYLKMQQHEQALADCRRALELDGQSVKAHFFLGQCQLEMESYDEA IANLQRAYSLAKEQRLNFGDDIPALRIAKKKRWNSIEERRIHQESSELH SYLSRLIAAERERELEECQRNHEGDEDDSHVRAQQACIEAKHDKYMADM DELFSQVDEKRKKRDIIPDYLCGKISFELMREPCITPSGITYDRKDIEEH LQRVGHFDPVTRSPLTQEQILIPNLAMKEVIDAFISENGWVEDY</p>
<u>Native sequence</u>	<p>Amino acids K2 – Y303 (end) of CHIP. Residue K232 of the fusion protein is equivalent to K2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 site of pGex6P-1

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**Nucleotide
Sequence**

ggatccAAGGGCAAGGAGGAGAAGGAGGGCGGCACGGCTGGGCGCTG
GCGGCGGAAGCCCCGAGAAGAGCCCCGAGCGCGCAGGAGCTCAAGGAGCA
GGCAATCGTCTGTTCGTGGGCCGAAAGTACCCGGAGGCGGCGGCCTGC
TACGGCCGCGCGATCACCCGGAACCCGCTGGTGGCCGTGTATTACACCA
ACCGGGCCTTGTGCTACCTGAAGATGCAGCAGCACGAGCAGGCCCTGGC
CGACTGCCGGCGCGCCCTGGAGCTGGACGGGCAGTCTGTGAAGGCGCAC
TTCTTCCTGGGGCAGTGCCAGCTGGAGATGGAGAGCTATGATGAGGCCA
TCGCCAATCTGCAGCGAGCTTACAGCCTGGCCAAGGAGCAGCGGCTGAA
CTTCGGGGACGACATCCCCAGCGTCTTCGAATCGCGAAGAAGAAGCGC
TGGAACAGCATTGAGGAGCGGCGCATCCACCAGGAGAGCGAGCTGCACT
CCTACCTCTCCAGGCTCATTGCCGCGGAGCGTGAGAGGGAGCTGGAAGA
GTGCCAGCGAAACCACGAGGGTGATGAGGACGACAGCCACGTCCGGGCC
CAGCAGGCCTGCATTGAGGCCAAGCACGACAAGTACATGGCGGACATGG
ACGAGCTTTTTTCTCAGGTGGATGAGAAGAGGAAGAAGCGAGACATCCC
CGACTACCTGTGTGGCAAGATCAGCTTTGAGCTGATGCGGGAGCCGTGC
ATCACGCCAGTGGCATCACCTACGACCGCAAGGACATCGAGGAGCACC
TGCAGCGTGTGGGTCATTTTGACCCCGTGACCCGGAGCCCCCTGACCCA
GGAACAGCTCATCCCCAACTTGGCTATGAAGGAGGTTATTGACGCATTC
ATCTCTGAGAATGGCTGGGTGGAGGACTACTgagcggccgc