

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of CHIP [2 - 303]

Enzyme description:- CHIP [2 – 303]

Clone number:- DU 3350

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni²⁺ -NTA agarose

Expression level:- 30 mg/L

Calculated molecular mass:-

Monoisotopic 38, 244.77 daltons
Average Mass 38, 268.91 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.16

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 3350
<u>Species</u>	Human
<u>Accession number</u>	NM_005852
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MGSSHHHHHSSGLVPRGSHMASMTGGQQMGRGSKGKEEKEGGARLGAG GGSPEKSPSAQELKEQGNRLFVGRKYPEAAACYGRAITRNPLVAVYYTN RALCYLKMQQHEQALADCRRALELDGQSVKAHFFLGQCQLEMESYDEAI ANLQRAYSLAKEQRLNFGDDIPALRIAKKKRWNSIEERRIHQESLHS YLSRLIAAERERELEECQRNHEGDEDDSHVRAQQACIEAKHDKYMADMD ELFSQVDEKRRKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHL QRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGVEDY
<u>Native sequence</u>	Amino acids K2 – Y303 (end) of CHIP. Residue K35 of the fusion protein is equivalent to K2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 14 - 19
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 site of pET28a

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**Nucleotide
Sequence**

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