CHIP [6His-tagged]

E3 Ligase

Alternate Names: C TERMINUS OF HSC70-INTERACTING PROTEIN; STUB1

Cat. No.	**63-0001-025**	Quantity:	25 µg
Lot. No.	**1382**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). C-Terminus of Hsc70 Interacting Protein (CHIP) is a member of the E3 protein ligase family and cloning of the human gene was first described by Ballinger *et al.* (1999). Human CHIP shares 97% and 53% amino acid identity with its mouse and Drosophila homologues respectively with the highest conservation in the 94 residues of the C-terminus. The intrinsic E3 ligase activity of CHIP is conferred through a Ubox domain at the C-terminus of the protein. CHIP interacts with the UBE2D E2 enzyme family targeting the Heat Shock Cognate protein-70 (HSC70) for ubiquitylation (Jiang *et al.*, 2001). Accumulation of PAELR a substrate for the E3 ligase Parkin occurs in the stressed endoplasmic reticulum (ER) causing neurodegeneration. Positive regulation of Parkin activity has been shown to occur through the dissociation of CHIP in complex with Parkin, HSP70 and PAELR in the ER, facilitating Parkin mediated PAELR ubiquitylation (Imai *et al.*, 2002). CHIP co-localises with α-synuclein in Lewy bodies and mediates alpha-synuclein degradation by both the proteasomal and lysosomal

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Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~38.3 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:

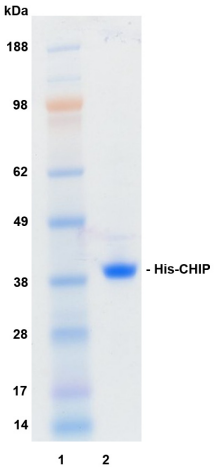
MGSSHHHHHHSSGLVPRGSHMASMTG
GQQMGRGS***K***GKEEKEGGARLGAGGGS
PEKSPSAQELKEQGNRLFVGRKYPEAAA
CYGRAITRNPLVAVYYTNRALCYLK
MQQHEQALADCRRALELDGQSVKAHFFL
GQCQLEMESYDEAIANLQRAYSLAKEQR
LNFGDDIPSALRIAKKKRWNSIEERRI
HQESELHSYLSRLIAAERERELEECQRN
HEGDEDDSHVRAQQACIEAKHDKYMADM
DELFSQVDEKRKKRDIPDYLCGKISFELM
REPCITPSGITYDRKDIEEHLQRVGHFD
PVTRSPLTQEQLIPNLAMKEVIDAFISENG
WVEDY

Tag (**bold text**): N-terminal His
Protease cleavage site: Thrombin (LVPR▼GS)
CHIP (regular text): Start ***bold italics*** (amino acid residues 2-303)
Accession number: NP_005852

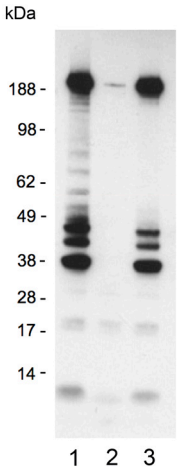
Quality Assurance

Protein Identification:
Confirmed by mass spectrometry.

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-CHIP



E3 ligase assay:
The ubiquitin conjugating activity of His-CHIP was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of His-CHIP for 30 minutes at 30˚C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or His-CHIP (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of ATP (lanes 1 and 3). Ubiquitin conjugates were detected in the absence of His-CHIP (lane 3) but they appeared to be more extensive in the presence of the E3 ligase.



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Lot-specific COA version tracker: v1.0.1

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pathways (Shin *et al.*, 2005). Cystic fibrosis arises from the misfolding and premature degradation of Cystic Fibrosis Transconductance Regulator (CFTR) carrying the deletion Phe508 (delF508). A cytosolic CHIP/Hsc70 complex cooperates with a ubiquitin ligase complex containing RMA1, UBE2J1, and derlin-1 to monitor the folding status of CFTR in the cytosol and target the mutant form (CFTR-DeltaF508) to the proteasome (Sha *et al.*, 2009; Younger *et al.*, 2006).

References:

Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY, Patterson C (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* **19**, 4535-45.

Imai Y, Soda M, Hatakeyama S, Akagi T, Hashikawa T, Nakayama KI, Takahashi R (2002) CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. *Mol Cell* **10**, 55-67.

Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C (2001) CHIP is a U-box-dependent E3 ubiquitin ligase: Identification of Hsc70 as a target for ubiquitylation. *J Biol Chem* **276**, 42938-44.

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Shin Y, Klucken J, Patterson C, Hyman BT, McLean PJ (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. *J Biol Chem* **280**, 23727-34.

Windheim M, Peggie M, Cohen P (2008) Two different classes of E2 ubiquitin-conjugating enzymes are required for the mono-ubiquitination of proteins and elongation by polyubiquitin chains with a specific topology. *Biochem J* **409**, 723-9.

Younger JM, Chen L, Ren HY, Rosser MF, Turnbull EL, Fan CY, Patterson C, Cyr DM (2006) Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell* **126**, 571-82.

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Lot-specific COA version tracker: v1.0.1