

# IDOL [6His-tagged]

## E3 Ligase

Alternate Names: MIR, MyLIP

Cat. No. 63-0044-025

Lot. No. 30229

Quantity: 25 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

## 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). Inducible Degradator of LDLR (IDOL) is a member of the E3 protein ligase family and cloning of the human gene was first described by Olsson *et al.* (1999). IDOL is a RING finger domain ubiquitin E3 ligase that is up-regulated by the sterol-activated transcription factors LXR alpha and LXR beta. IDOL activity leads to ubiquitylation and degradation of the low density lipoprotein (LDL) receptor (LDLR). LDLR is essential for the uptake of LDL cholesterol and the regulation of plasma lipoprotein levels and lipid homeostasis (Zelcer *et al.*, 2009; Zhang *et al.*, 2011). An inherited loss-of-function mutation in the LDLR gene in humans or poor diet can elevate plasma LDL levels, reduce LDL clearance and accelerate atherosclerosis and the risk of cardiovascular disease (Tolleshaug *et al.*, 1983; Brown and Goldstein 1986).

### References:

Brown MS, Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* **232**, 34-47.

Olsson PA, Korhonen L, Mercer EA, Lindholm D (1999) MIR is a novel ERM-like protein that interacts with myosin regulatory light chain and inhibits neurite outgrowth. *J Biol Chem* **274**, 36288-36292.

Tolleshaug H, Hobgood KK, Brown MS, Goldstein JL (1983) The LDL receptor locus in familial hypercholesterolemia: multiple mutations disrupt transport and processing of a membrane receptor. *Cell* **32**, 941-51.

Continued on page 2

## Physical Characteristics

**Species:** human

**Source:** *E. coli*

**Quantity:** 25 µg

**Concentration:** 1.0 mg/ml

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

**Molecular Weight:** ~54 kDa

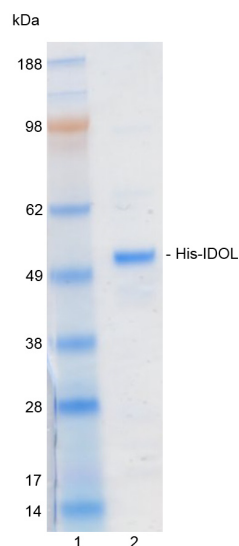
**Purity:** >80% by InstantBlue™ SDS-PAGE

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

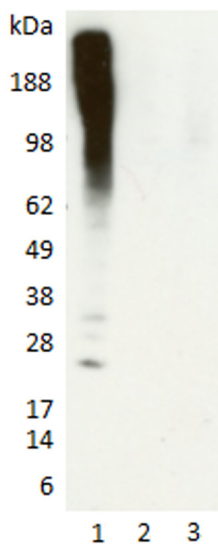
**Protein Sequence:** Please see page 2

## Quality Assurance

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



**Protein Identification:** Confirmed by mass spectrometry.



**E3 ligase assay:** The ubiquitin conjugating activity of His-IDOL was validated through its ability to catalyze the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D2 (UbcH5b) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of His-IDOL for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D2 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or His-IDOL (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an HRP-linked streptavidin conjugate and these were observed only in the presence of both ATP and His-IDOL.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

## Background

Continued from page 1

Zelcer N, Hong C, Boyadjian R, Tontonoz P (2009) LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science* 325, 100-4.

Zhang L, Fairall L, Goult BT, Calkin AC, Hong C, Millard CJ, Tontonoz P, Schwabe JW (2011) The IDOL-UBE2D complex mediates sterol-dependent degradation of the LDL receptor. *Genes Dev* 25, 1262-74.

## Physical Characteristics

Continued from page 1

### Protein Sequence:

**MGSSHHHHSSGLVPRGSHMASMTG**  
**GOQMGRGSENLYFQGM**LCYVTRPDAV  
LMEVEVEAKANGEDCLNQCRRLLGIEVDYF  
GLQFTGSKGESLWLNLRNRISQQMDG  
LAPYRLKLRVKFFVEPHLILQEQTRHIF  
FLHIKEALLAGHLCSPEQAVELSAL  
LAQTKFGDYNQNTAKYNYEELCAKELSS  
ATLNSIVAKHKELEGTSQASAEYQVLQI  
VSAMENYGI EWHSVRDSEGQKLLIGVG  
PEGISICKDDFSPINRIAYPVVQMATQS  
GKNVYLTVTKESGNSIVLLFKMISTRAAS  
GLYRAITETHAFYRCDTVTSVMMQYSRDLK  
GHLASLFLNENINLGKKYVFDIKRTSKEV  
YDHARRALYNAGVVDLVSRRNQSPSH  
SPLKSESSMNCSSCEGLSCQOTRVLQEKL  
RKLKEAMLCMVCEEEINSTFCPCGHTVC  
CESCAAQLQSCPVCRSRVEHVQHVYLPHTHT  
SLLNLTVI

Tag (**bold text**): N-terminal 6His  
Protease cleavage site: TEV™ (ENLYFQ▼G)  
IDOL (regular text): Start **bold italics** (amino acid residues 1-445)  
Accession number: NP\_037394.2



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