

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

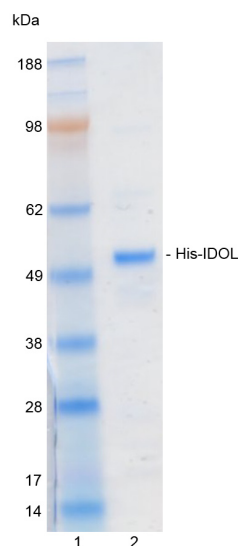
Quality Assurance

Purity: 4-12% gradient SDS-PAGE

InstantBlue™ staining

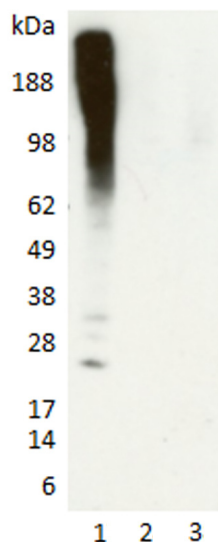
Lane 1: MW markers

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.



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

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 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:

MGSSHHHHHSSGLVPRGSHMASMTG
GOQMGRGSENLYFQGMLCYVTRPDAV
LMEVEVEAKANGEDCLNQCRRLLGIEVDYF
GLQFTGSKGESLWLNLRNRISQQMDG
LAPYRLKLRVKFFVEPHLILQEQTRHIF
FLHIKEALLAGHLCSPEQAVELSAL
LAQTKFGDYNQNTAKYNYEELCAKELSS
ATLNSIVAKHKELEGTSQASAEYQVLQI
VSAMENYGI EWHSVRDSEGQKLLIGVG
PEGISICKDDFSPINRIAYPVVQMATQS
GKNVYLTVTKESGNSIVLLFKMISTRAAS
GLYRAITETHAFYRCDTVTSAVMMQYSRDLK
GHLASLFLNENINLGKKYVFDIKRTSKEV
YDHARRALYNAGVVDLVSRRNQSPSH
SPLKSESSMNCSSCEGLSCQOTRVLQEKL
RKLKEAMLCMVCEEEINSTFCPCGHTVC
CESCAAQLQSCPVCRSRVEHVQHVYLPHTT
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



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0