

## Certificate of Analysis

### Idol (c-Myc tagged), active

(Recombinant E3 ligase expressed in Sf21 insect cells)

Item # 23-064, 23-064-K, 23-064M

Parent Lot # D12NP009N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** N-terminal c-Myc, 6His-tagged, recombinant human Idol full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 83% by SDS-PAGE and Coomassie blue staining. MW = 55kDa.

**Formulation:** 1.186mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Activity (Parent lot# D12NP009N):** This lot of Idol (c-Myc tagged), active is active and meets product specifications.

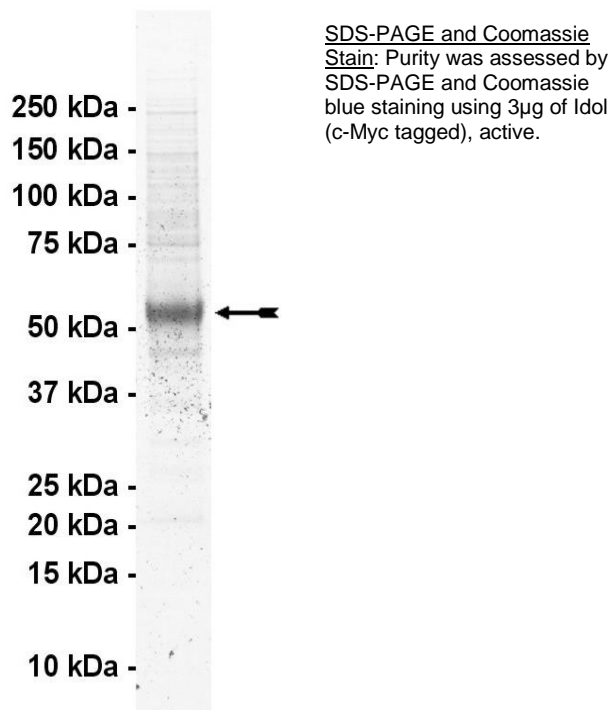
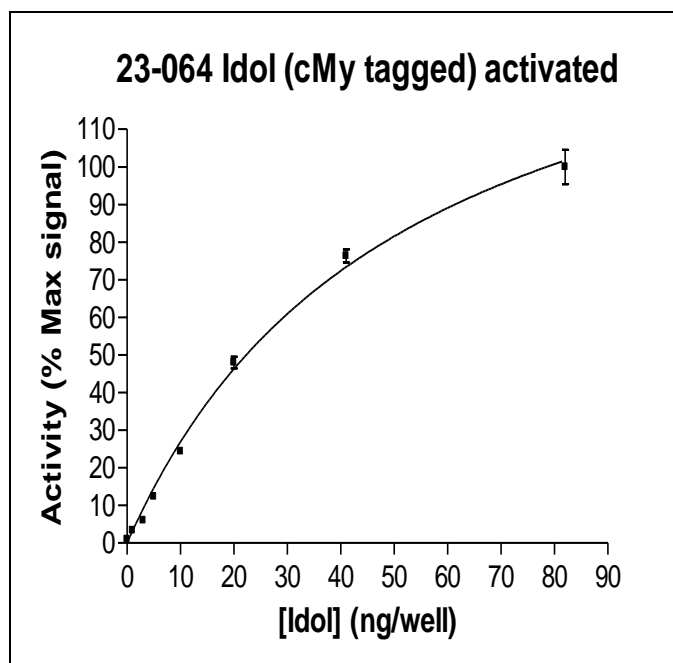
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Assay:** This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

**Protein Identity:** Confirmed identity as Idol by mass spectrometry.



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### E3 Assay Protocol

#### Reagents:

- |  |                           |
|--|---------------------------|
| 1. UBE1, active (Item # 23-021)                | 4. 1x Reaction Buffer     |
| 2. UbcH4, active (Item # 23-025)               | 5. Biotinylated-Ubiquitin |
| 3. Idol (c-Myc tagged), active (Item # 23-064) | 6. Stop Solution          |

#### Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>).

Idol (c-Myc tagged) active is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 10μM ATP, 10nM UBE1, 100nM UbcH4 and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH 7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Idol (c-Myc tagged), active activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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### Idol (c-Myc tagged), active Information

**Protein** human Idol

**Accession number** GenBank BC002860

**Alternative Names** E3 ubiquitin-protein ligase MYLIP, Inducible degrader of the LDL-receptor (Idol), Myosin regulatory light chain interacting protein (MIR)

### **Key Facts**

The low density lipoprotein receptor (LDLR) mediates the uptake of LDL into cells and is considered a crucial determinant of cholesterol metabolism in the body. Elevated levels of LDL-cholesterol in the plasma are recognized as a critical predictor of atherosclerosis and coronary heart disease.

Idol is a RING finger domain ubiquitin ligase that is up-regulated by the sterol-responsive nuclear receptor LXR and mediates the ubiquitination of the cytoplasmic domain of LDLR, promoting proteasomal degradation. Cells lacking Idol exhibit markedly elevated levels of the LDLR protein and increased rates of LDL uptake.

Idol has also been shown to target the closely related LDLR family members VLDLR (very low density lipoprotein receptor) and ApoER2 (ApoE receptor 2), proteins that are implicated in neuronal development and lipid metabolism.

**Related Products** Item # 23-021 UBE1, active, Item # 23-025 UbchH4, active, Item # 23-062 Idol, active, Item # 23-063 VLDLR

### **Selected References**

Zelcer N. *et al.*, LXR Regulates Cholesterol Uptake through Idol-dependent Ubiquitination of the LDL Receptor. *Science* 325: 100-104, 2009

Hong C. *et al.*, The E3 Ubiquitin Ligase Idol Induces the Degradation of the Low Density Lipoprotein Receptor Family Members VLDLR and ApoER2. *J Biol Chem.* 285: 19720–19726, 2010

Zhang L. *et al.*, The Idol–UBE2D Complex Mediates Sterol-Dependent Degradation of the LDL Receptor. *Genes Dev.* 25: 1262-1274, 2011

Scotti E. *et al.*, Targeted Disruption of the Idol Gene Alters Cellular Regulation of the Low-Density Lipoprotein Receptor by Sterols and Liver X Receptor Agonists. *Mol Cell Biol.* 31: 1885-1893, 2011

Sorrentino V. *et al.*, Distinct Functional Domains Contribute to Degradation of the Low Density Lipoprotein Receptor (LDLR) by the E3 Ubiquitin Ligase Inducible Degradator of the LDLR (IDOL). *J Biol Chem.* 286: 30190–30199, 2011

Reviewed and approved by site quality representative.

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