UBE2M (mouse; full length), pAb

Alternate Names: Nedd8-conjugating enzyme, Ubc12, UBC-RS2, UBC12.

Cat. No.	**68-0025-100**	Quantity:	100 µg
Lot. No.	**30262**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

The enzymes of the NEDDylation pathway play a pivotal role in a number of cellular processes including the indirect regulation and targeting of substrate proteins for proteasomal degradation. Three classes of enzymes are involved in the process of NEDDylation; the ubiquitin-like activating enzyme APP-BP1/Uba3 (E1), the ubiquitin-like conjugating enzymes (E2s) and protein ligases (E3s). UBE2M is a member of the E2 conjugating enzyme family and the gene for human UBE2M was first described by Osaka *et al.* (1998) and shares 42% sequence identity with yeast UBE2M. A trapped ubiquitin like activation complex has been described for the NEDD8 pathway comprising, the E1 APP-BP1/Uba3, two NEDD8 molecules, UBE2M and MgATP. Thioester linkage of NEDD8 to APP-BP1-Uba3 results in an alternate E1 conformation that exposes two NEDD8 binding sites on the E2 enzyme. After transfer of the non-covalently bound NEDD8 to the E2, an alternate E1 conformation allows the release of the thioester bound NEDD8 product. Transfer of the NEDD8 thioester linkage between E1 and E2 enzymes in this way can induce a conformational change and alter downstream signalling in the NEDD8 ubiquitin-like (Ubl) cascade (Huang *et al.*, 2007). The interaction of different E2 enzymes with

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: mouse Ube2M (residues 1-183) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Ube2M at ~22 kDa

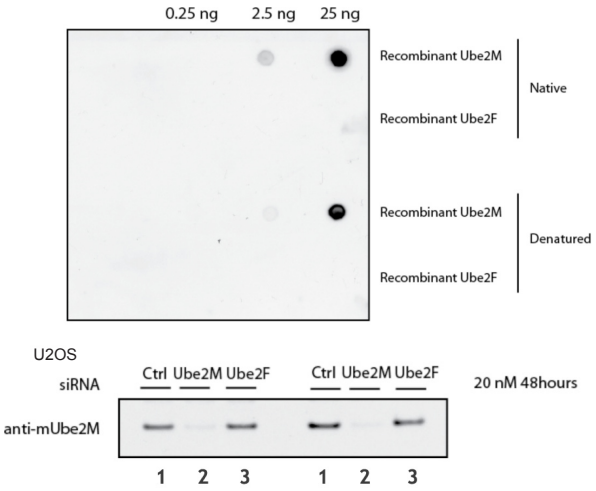
Reactivity: mouse; other species not tested.

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.5 µg/ml

Immunoprecipitation:
Not tested



Dot Blotting Analysis:
By dot blot assay the specific recognition of recombinant Ube2M protein was observed under native and denaturing conditions when probed with 0.5µg/ml anti-Ube2M antibody (Cat# 68-0025-100). No cross reactivity was observed with Ube2F.

Western Blotting Analysis:
U2OS cells were transfected with either control siRNA, Ube2M siRNA or Ube2F siRNA (lanes 1, 2 and 3 respectively). By Western blotting the specific recognition of a band corresponding to Ube2M was observed in lysates treated with control siRNA (lane 1) or Ube2F siRNA (lane 3) compared to lysates treated with Ube2M siRNA (lane 2) where the presence of Ube2M could not be detected when probed with 0.5 µg/ml anti-Ube2M antibody (Cat# 68-0025-100).

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Lot-specific COA version tracker: v1.0.0

UBE2M (mouse; full length), pAb

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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

different Cullin RING E3 Ligases (CRLs) has been determined; for example UBE2M/RBX1 and UBE2F/RBX2 can interact with such ligases. This reveals the functional importance of hierarchical expansion of the NEDD8 conjugation system in establishing selective CRL activation (Huang *et al.*, 2009). Following ionizing irradiation of a human oesophageal cancer cell line a cDNA microarray screen found UBE2M to be up-regulated suggesting a role for UBE2M in oesophageal cancer (Bo *et al.*, 2004).

Antibody Production:
Anti-UBE2M (mouse) polyclonal antibody was raised in sheep against UBE2M (residues 1-183 of mouse UBE2M). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-UBE2M pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-UBE2M (mouse) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Bo H, Ghazizadeh M, Shimizu H, Kurihara Y, Egawa S, Moriyama Y, Tajiri T, Kawanami O (2004) Effect of ionizing irradiation on human esophageal cancer cell lines by cDNA microarray gene expression analysis. *J Nippon Med Sch* **71**, 172-80.

Huang DT, Ayrault O, Hunt HW, Taherbhoy AM, Duda DM, Scott DC, Borg LA, Neale G, Murray PJ, Roussel MF, Schulman BA (2009) E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. *Mol Cell* **33**, 483-95.

Huang DT, Hunt HW, Zhuang M, Ohi MD, Holton JM, Schulman BA (2007) Basis for a ubiquitin-like protein thioester switch toggling E1-E2 affinity. *Nature* **445**, 394-8.

Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, Kawashima S, Tanaka K, Kato S (1998) A new NEDD8-ligating system for cullin-4A. *Genes Dev* **12**, 2263-8.

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Lot-specific COA version tracker: v1.0.0