# abcam

### Product datasheet

## Anti-UBE2M/UBC12 antibody [EPR5333] ab109507

Recombinant RabMAb

#### 10 References 6 Images

#### Overview

**Product name** Anti-UBE2M/UBC12 antibody [EPR5333]

**Description** Rabbit monoclonal [EPR5333] to UBE2M/UBC12

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK-293T and Ramos cell lysates. IHC-P: Human colonic adenocarcinoma and lung

carcinoma tissues. ICC/IF: Ramos cells.

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### **Properties**

**Form** Liquid

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

**Purity** Protein A purified

Clonality Monoclonal Clone number **EPR5333** 

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab109507 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/100 - 1/1000. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/10000 - 1/50000. Detects a band of approximately 20 kDa (predicted molecular weight: 21 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/250.

Function Accepts the ubiquitin-like protein NEDD8 from the UBA3-NAE1 E1 complex and catalyzes its

covalent attachment to other proteins. The specific interaction with the E3 ubiquitin ligase RBX1,

but not RBX2, suggests that the RBX1-UBE2M complex neddylates specific target proteins, such

as CUL1, CUL2, CUL3 and CUL4. Involved in cell proliferation.

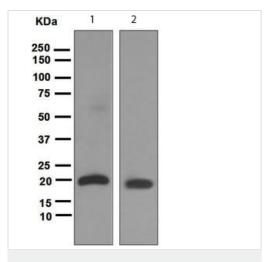
Pathway Protein modification; protein neddylation.

**Sequence similarities** Belongs to the ubiquitin-conjugating enzyme family. UBC12 subfamily.

**Domain**Both the N-terminal docking peptide and the catalytic core domain must bind the UBA3-NAE1

complex simultaneously for optimal transfer of NEDD8.

#### **Images**



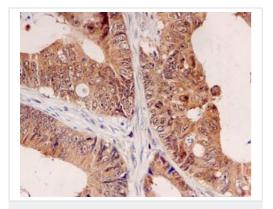
Western blot - Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507)

**All lanes :** Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507) at 1/10000 dilution

Lane 1 : HEK-293T cell lysate
Lane 2 : Ramos cell lysate

Lysates/proteins at 10 µg per lane.

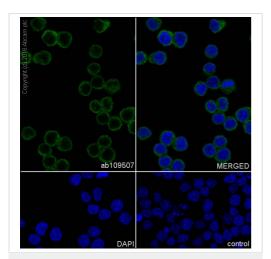
**Predicted band size:** 21 kDa **Observed band size:** 20 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507)

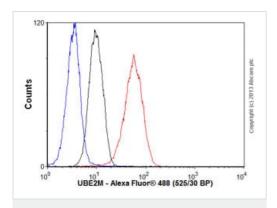
Immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using ab109507 at a dilution of 1/100.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



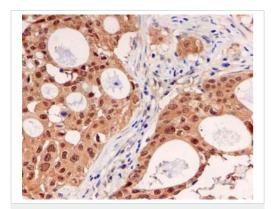
Immunocytochemistry/ Immunofluorescence - Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507)

Immunocytochemistry/ Immunofluorescence analysis of Ramos (human Burkitt's lymphoma B lymphocyte) cells labeling UBE2M/UBC12 with purified ab109507 at 1/250 dilution (8  $\mu$ g/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2  $\mu$ g/mL) dilution. DAPI (blue) was used as nuclear counterstain.



Flow Cytometry (Intracellular) - Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507)

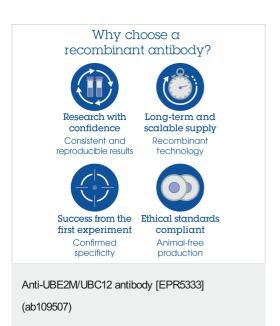
Overlay histogram showing Ramos cells stained with ab109507 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109507, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor<sup>®</sup> 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in Ramos cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-UBE2M/UBC12 antibody [EPR5333] (ab109507)

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue using ab109507 at a dilution of 1/100.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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