abcam

Product datasheet

Anti-MCL1 antibody [Y37] ab32087

KO 評価済 リコンビナント RabMAb

★★★★★ 14 Abreviews 138 References 画像数 10

製品の概要

製品名	Anti-MCL1 antibody [Y37]
製品の詳細	Rabbit monoclonal [Y37] to MCL1
由来種	Rabbit
特異性	This antibody recognises MCL1. The antibody does not cross-react with other Bcl-2 family members.
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human MCL1 aa 100-200. The exact sequence is proprietary. Database link: **Q07820** (Peptide available as **ab199979**)
ポジティブ・コントロール	WB: Human lung, lung cancer and liver lysates; HEK293T, A431, Ramos, H:-60, HeLa, MCF7 and HepG2 whole cell lysate (**ab7900**). IHC-P: Human colon adenocarcinoma tissue. ICC/IF: HCT 116, MCF7 and H1299 cells. Flow Cyt (intra): Ramos and A431 cells.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y37
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab32087の使用に適用されます

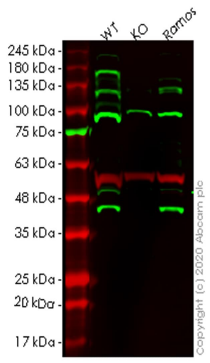
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/250. For unpurified use 1 ug for 106 cells. (For lot-specific stock concentration, please contact Abcam).**ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ **(2)**	1/100 - 1/500.
WB	★★★★★ **(10)**	1/1000 - 1/5000. Predicted molecular weight: 37 kDa.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

ターゲット情報

機能	Involved in the regulation of apoptosis versus cell survival, and in the maintenance of viability but not of proliferation. Mediates its effects by interactions with a number of other regulators of apoptosis. Isoform 1 inhibits apoptosis. Isoform 2 promotes apoptosis.
配列類似性	Belongs to the Bcl-2 family.
翻訳後修飾	Cleaved by CASP3 during apoptosis. In intact cells cleavage occurs preferentially after Asp-127, yielding a pro-apoptotic 28 kDa C-terminal fragment. Rapidly degraded in the absence of phosphorylation on Thr-163 in the PEST region. Phosphorylated on Thr-163. Treatment with taxol or okadaic acid induces phosphorylation on additional sites.
細胞内局在	Membrane. Cytoplasm. Mitochondrion. Nucleus > nucleoplasm. Cytoplasmic, associated with mitochondria.

画像



Western blot - Anti-MCL1 antibody [Y37] (ab32087)

All lanes : Anti-MCL1 antibody [Y37] (ab32087) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : MCL1 knockout HEK293T cell lysate
Lane 3 : Ramos cell lysate

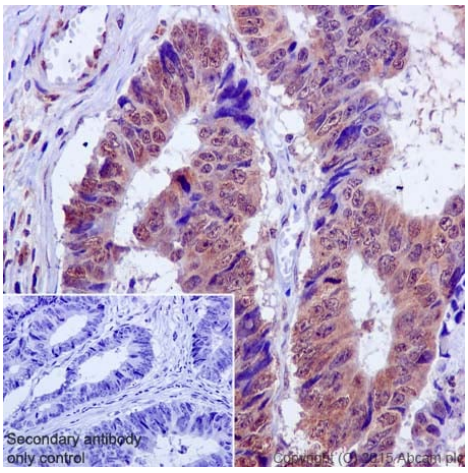
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 37 kDa
Observed band size: 37 kDa

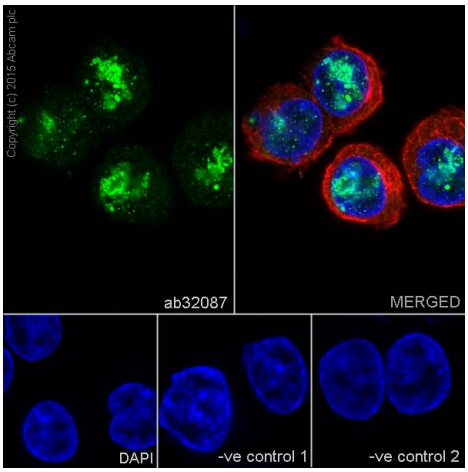
Lanes 1-3: Merged signal (red and green). Green - ab32087 observed at 37 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab32087 Anti-MCL1 antibody [Y37] was shown to specifically react with MCL1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266838** (knockout cell lysate **ab256986**) was used. Wild-type and MCL1 knockout samples were subjected to SDS-PAGE. ab32087 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MCL1 antibody [Y37] (ab32087)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon adenocarcinoma tissue labelling MCL1 with purified ab32087 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

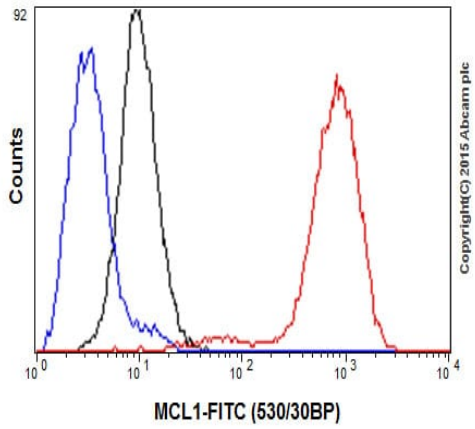


Immunocytochemistry/ Immunofluorescence - Anti-MCL1 antibody [Y37] (ab32087)

Immunocytochemistry/Immunofluorescence analysis of HCT 116 (human colorectal carcinoma cell line) cells labelling MCL1 with purified ab32087 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

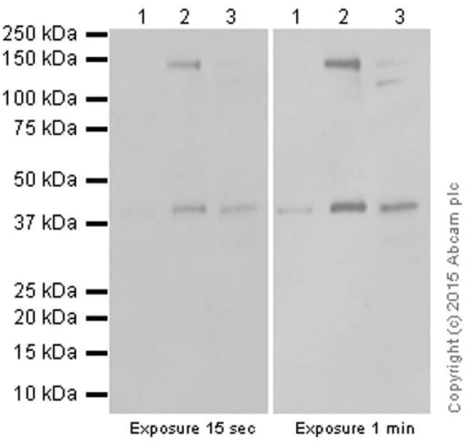
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Flow Cytometry (Intracellular) - Anti-MCL1 antibody [Y37] (ab32087)

Intracellular Flow Cytometry analysis ofRamos (human Burkitt's lymphoma cell line) cells labelling MCL1 with purified ab32087 at 1/250 (red). Cells were fixed with 4% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-MCL1 antibody [Y37] (ab32087)

All lanes : Anti-MCL1 antibody [Y37] (ab32087) at 1/1000 dilution

Lane 1 : Human lung tissue with NFDM/TBST
Lane 2 : Human lung cancer tissue with NFDM/TBST
Lane 3 : Human liver tissue with NFDM/TBST

Lysates/proteins at 20 µg per lane.

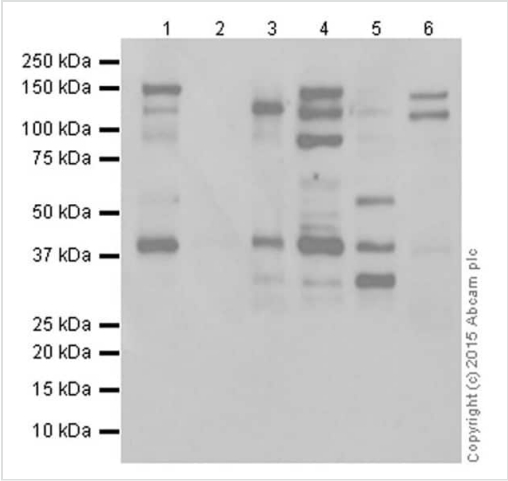
Blocking peptides at 5 % per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (H+L), Peroxidase conjugated. at 1/1000 dilution

Predicted band size: 37 kDa

Exposure time for samples 1-3: 15 seconds; exposure time for samples 4-6: 1 minute.

Additional bands: We are unsure as to the identity of these extra bands.



Western blot - Anti-MCL1 antibody [Y37] (ab32087)

All lanes : Anti-MCL1 antibody [Y37] (ab32087) at 1/1000 dilution

Lane 1 : Ramos (human Burkitt's lymphoma cell line) cell lysate with NFDM/TBST
Lane 2 : HL-60 (human promyelocytic leukemia cell line) cell lysate with NFDM/TBST
Lane 3 : A431 (human epidermoid carcinoma cell line) cell lysate with NFDM/TBST
Lane 4 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate with NFDM/TBST
Lane 5 : MCF7 (human breast adenocarcinoma cell line) cell lysate with NFDM/TBST
Lane 6 : HepG2 (human liver hepatocellular carcinoma cell line) cell lysate with NFDM/TBST

Lysates/proteins at 20 µg per lane.

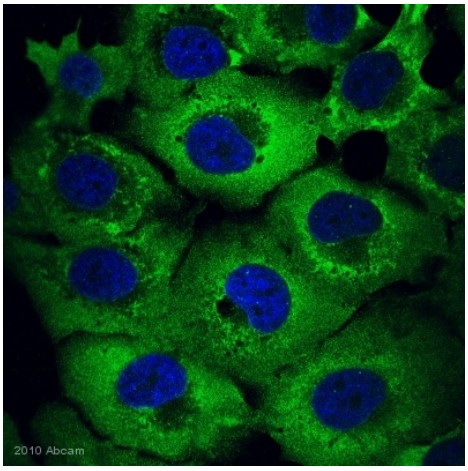
Blocking peptides at 5 % per lane.

Secondary
All lanes : Goat anti-rabbit IgG (H+L), peroxidase conjugated. at 1/1000 dilution

Predicted band size: 37 kDa

Exposure time: 15 seconds

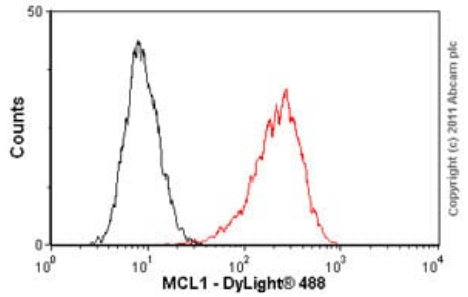
Additional bands: We are unsure as to the identity of these extra bands.



Immunocytochemistry/ Immunofluorescence - Anti-MCL1 antibody [Y37] (ab32087)

This image is courtesy of an anonymous Abreview.

Immunocytochemistry/Immunofluorescence analysis of H1299 cells labelling MCL1 with unpurified ab32087. Cells were PFA-fixed and permeabilized in 0.5% Triton X-100 prior to blocking in 3% Serum for 1 hour at 24°C. The primary antibody was diluted 1/100 and incubated with the sample for 1 hour at 24°C. The secondary antibody was an Alexa Fluor® 488-conjugated Goat anti-Rabbit polyclonal, diluted 1/2000. DAPI (blue) was used as the nuclear counterstain.

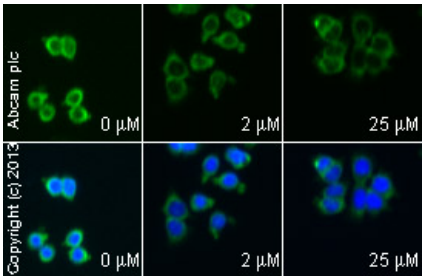


Flow Cytometry (Intracellular) - Anti-MCL1 antibody [Y37] (ab32087)

Intracellular Flow Cytometry analysis ofA431 (human epidermoid carcinoma cell line) cells labelling MCL1 with unpurified ab32087 (red line). Cells were fixed with methanol (5 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 (ab32087, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C.

Black - Isotype control, rabbit monoclonal IgG.

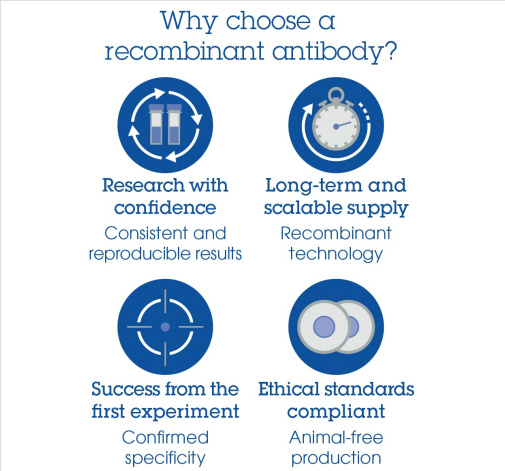
Acquisition of >5,000 events was performed. This antibody gave a decreased signal in A431 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-MCL1 antibody [Y37] (ab32087)

Immunocytochemistry/Immunofluorescence analysis of HCT 116 (human colorectal carcinoma cell line) cells treated with wogonin (**ab142471**) labelling MCL1 with unpurified ab32087. Decrease of MCL1 expression correlates with increased concentration of wogonin, as described in literature. Cells were incubated at 37°C for 2h in media containing different concentrations of **ab142471** (wogonin) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab32087 (1/100) dilution was performed overnight at 4°C in PBS containing 1% BSA

and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.



Anti-MCL1 antibody [Y37] (ab32087)

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