abcam

Product datasheet

Anti-YB1 antibody [EP2708Y] ab76149

リコンビナント RabMAb

★★★★★ 3 Abreviews 60 References 画像数 16

製品の概要

製品名	Anti-YB1 antibody [EP2708Y]
製品の詳細	Rabbit monoclonal [EP2708Y] to YB1
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human YB1 aa 250 to the C-terminus (C terminal). The exact sequence is proprietary. (Peptide available as **ab175051**)
ポジティブ・コントロール	WB: HeLa, SW480, A549, C6 PC-12, NIH/3T3, Raw264.7 and MCF7 cell lysates. IHC-P: Human kidney, human cervical carcinoma, mouse liver and rat stomach tissues. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HEK293, HeLa and MCF-7 whole cell lysate.
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 (glycerin, glycerine), 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	EP2708Y
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ab76149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。

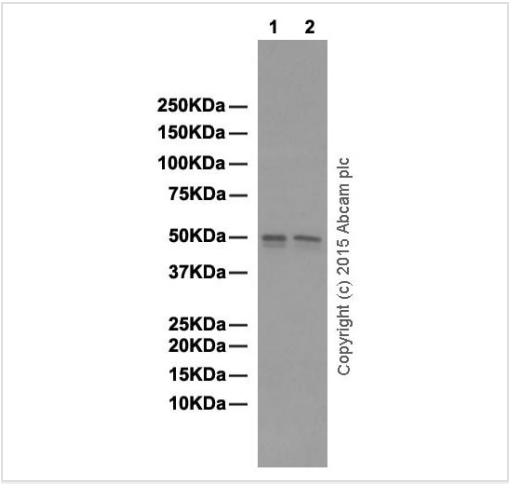
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/50 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (2)	1/100. **For unpurified use at 5µg/ml.**
WB	★★★★★ (1)	1/1000. Predicted molecular weight: 36 kDa. **For unpurified use at 1/10000 - 1/20000.**
IP		1/30.
IHC-P		1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

ターゲット情報

機能	Mediates pre-mRNA alternative splicing regulation. Binds to splice sites in pre-mRNA and regulates splice site selection. Binds and stabilizes cytoplasmic mRNA. Contributes to the regulation of translation by modulating the interaction between the mRNA and eukaryotic initiation factors (By similarity). Regulates the transcription of numerous genes. Its transcriptional activity on the multidrug resistance gene MDR1 is enhanced in presence of the APEX1 acetylated form at 'Lys-6' and 'Lys-7'. Binds to promoters that contain a Y-box (5'-CTGATTGGCCAA-3'), such as MDR1 and HLA class II genes. Promotes separation of DNA strands that contain mismatches or are modified by cisplatin. Has endonucleolytic activity and can introduce nicks or breaks into double-stranded DNA (in vitro). May play a role in DNA repair. Component of the CRD-mediated complex that promotes MYC mRNA stability. The secreted form acts as an extracellular mitogen and stimulates cell migration and proliferation.
配列類似性	Contains 1 CSD (cold-shock) domain.
翻訳後修飾	Ubiquitinated by RBBP6; leading to a decrease of YBX1 transcativational ability. In the absence of phosphorylation the protein is retained in the cytoplasm. Cleaved by a 20S proteasomal protease in response to agents that damage DNA. Cleavage takes place in the absence of ubiquitination and ATP. The resulting N-terminal fragment accumulates in the nucleus.
細胞内局在	Cytoplasm. Nucleus. Cytoplasmic granule. Secreted. Localized in cytoplasmic mRNP granules containing untranslated mRNAs. Shuttles between nucleus and cytoplasm. Predominantly cytoplasmic in proliferating cells. Cytotoxic stress and DNA damage enhance translocation to the nucleus. Localized with DDX1, MBNL1 and TIAL1 in stress granules upon stress. Secreted by

mesangial and monocytic cells after inflammatory challenges. Translocates from the cytoplasm to the nucleus after and colocalizes with APEX1 in nuclear speckles after genotoxic stress.

画像



Western blot - Anti-YB1 antibody [EP2708Y] (ab76149)

All lanes : Anti-YB1 antibody [EP2708Y] (ab76149) at 1/1000 dilution (purified)

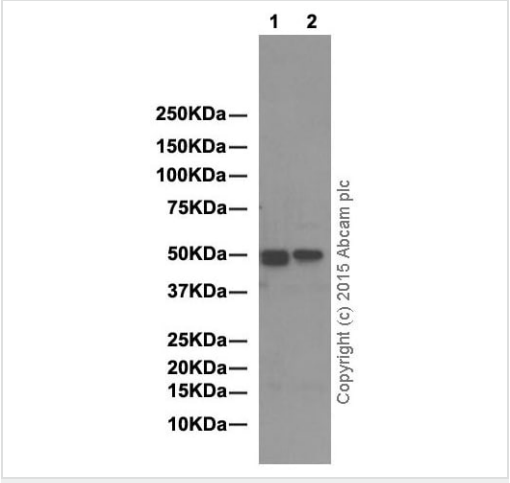
Lane 1 : NIH/3T3 whole cell lysate
Lane 2 : Raw264.7 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 36 kDa
Observed band size: 50 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-YB1 antibody [EP2708Y] (ab76149)

All lanes : Anti-YB1 antibody [EP2708Y] (ab76149) at 1/10000 dilution (purified)

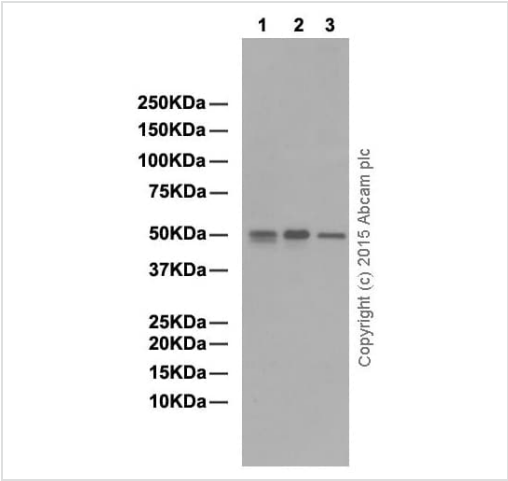
Lane 1 : C6 whole cell lysate
Lane 2 : PC-12 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 36 kDa
Observed band size: 50 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-YB1 antibody [EP2708Y] (ab76149)

All lanes : Anti-YB1 antibody [EP2708Y] (ab76149) at 1/1000 dilution (purified)

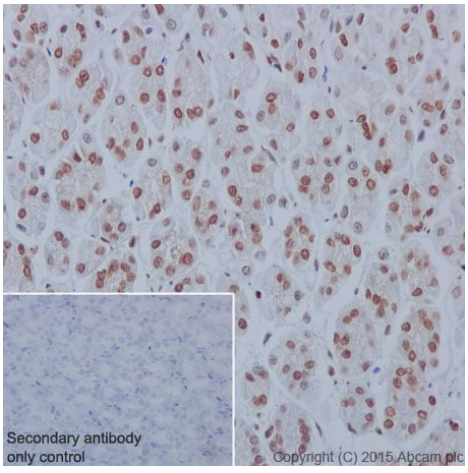
Lane 1 : HeLa whole cell lysate
Lane 2 : SW480 whole cell lysate
Lane 3 : A549 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

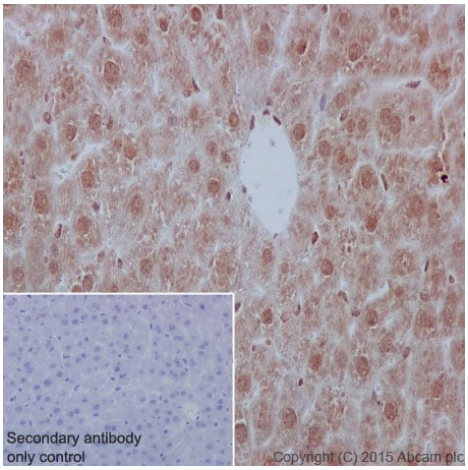
Predicted band size: 36 kDa
Observed band size: 50 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



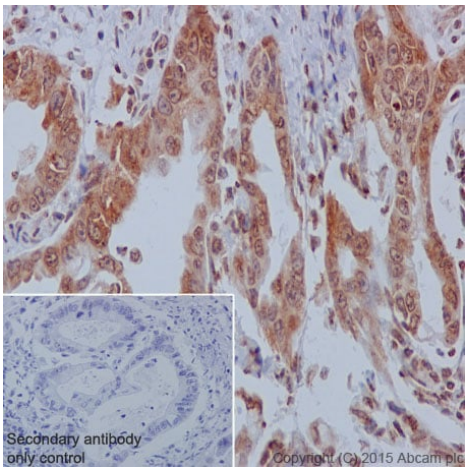
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat stomach tissue labelling YB1 with purified ab76149 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



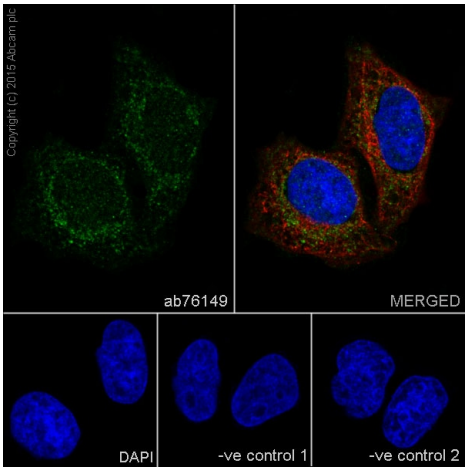
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue labelling YB1 with purified ab76149 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling YB1 with purified ab76149 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

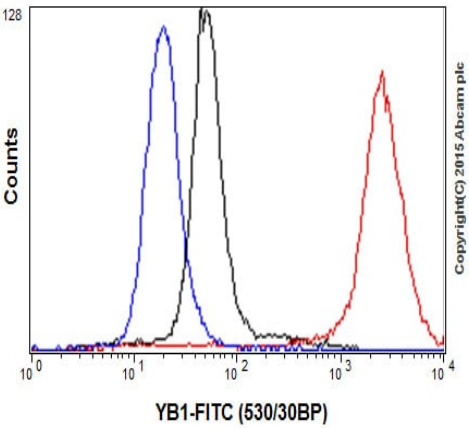


Immunocytochemistry/ Immunofluorescence - Anti-YB1 antibody [EP2708Y] (ab76149)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling YB1 with purified ab76149 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) 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.

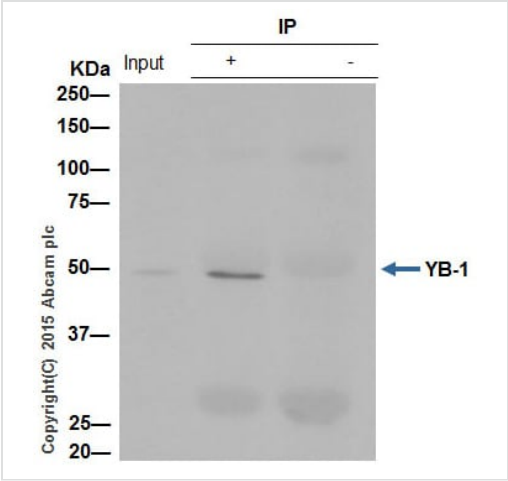
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

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



Flow Cytometry (Intracellular) - Anti-YB1 antibody [EP2708Y] (ab76149)

Intracellular Flow Cytometry analysis of HeLa cells labelling YB1 with purified ab76149 at 1/90 (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.



Immunoprecipitation - Anti-YB1 antibody [EP2708Y] (ab76149)

ab76149 (purified) at 1/30 immunoprecipitating YB1 in MCF-7 whole cell lysate.

Lane 1 (input): MCF-7 whole cell lysate (10µg)

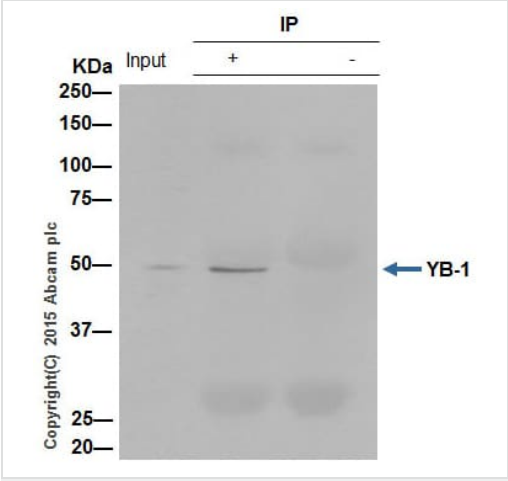
Lane 2 (+): ab76149 + MCF-7 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab76149 in MCF-7 whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunoprecipitation - Anti-YB1 antibody [EP2708Y] (ab76149)

ab76149 (purified) at 1/30 immunoprecipitating YB1 in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)

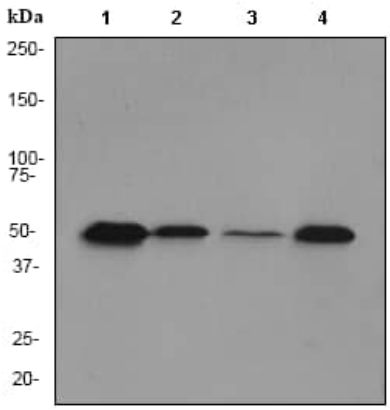
Lane 2 (+): ab76149 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab76149 in HeLa whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-YB1 antibody [EP2708Y] (ab76149)

All lanes : Anti-YB1 antibody [EP2708Y] (ab76149) at 1/200000 dilution (unpurified)

Lane 1 : HeLa cell lysate
Lane 2 : SW480 cell lysate
Lane 3 : A549 cell lysate
Lane 4 : MCF7 cell lysate

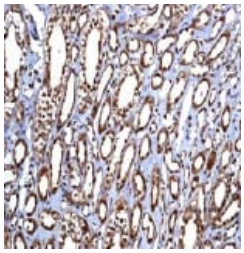
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/1000 dilution

Predicted band size: 36 kDa
Observed band size: 50 kDa

Immunocytochemistry/ Immunofluorescence - Anti-YB1 antibody [EP2708Y] (ab76149)

ICC/IF image of unpurified ab76149 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab76149, 5µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h.Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



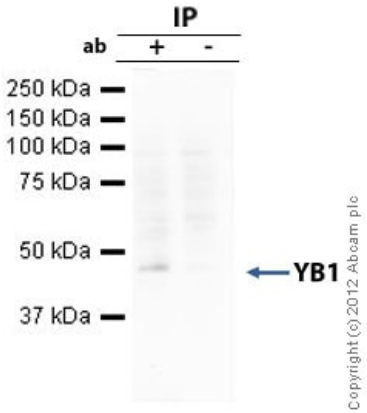
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling YB1 with unpurified ab76149 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Flow Cytometry (Intracellular) - Anti-YB1 antibody [EP2708Y] (ab76149)

Overlay histogram showing HeLa cells stained with unpurified ab76149 (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 (unpurified ab76149, 1/100 dilution) 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. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with methanol (5 min)/permeabilized with 0.1% PBS-Tween 20 used under the same conditions.



Immunoprecipitation - Anti-YB1 antibody [EP2708Y] (ab76149)

YB1 was immunoprecipitated using 0.5mg HEK293 whole cell extract, 10µg of Rabbit monoclonal to YB1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, HEK293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with unpurified ab76149.
Secondary: Mouse monoclonal [SB62a] secondary antibody to rabbit IgG light chain (HRP) (**ab99697**).
Band: 46kDa: YB1.

Anti-YB1 antibody [EP2708Y] (ab76149)

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