abcam

Product datasheet

Anti-YB1 antibody [EP2708Y] ab76149

重组 RabMAb

★★★★★ 2 Abreviews 18 References 15 图像

概述

产品名称	Anti-YB1抗体[EP2708Y]
描述	兔单克隆抗体[EP2708Y] to YB1
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, WB, IP, IHC-P, Flow Cyt
种属反应性	**与反应:** 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)
阳性对照	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: HeLa cells. IP: HEK293, HeLa and MCF-7 whole cell lysate.
常规说明	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EP2708Y
同种型	IgG

应用

Our Abpromise guarantee covers the use of **ab76149** in the following tested applications.

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

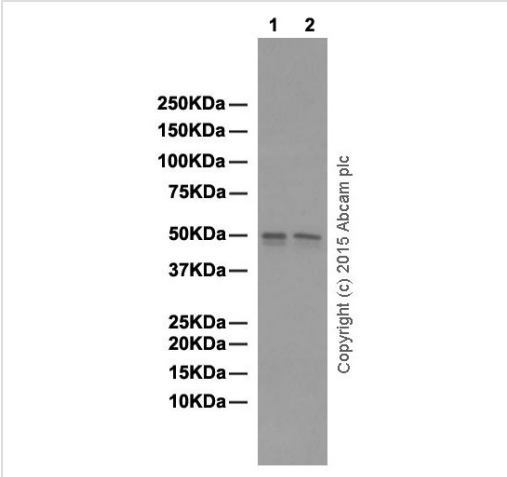
应用	Ab评论	说明
ICC/IF	★★★★★	1/100. **For unpurified use at 5µg/ml.**
WB	★★★★★	1/1000. Predicted molecular weight: 36 kDa.Can be blocked with YB1 peptide (ab175051). **For unpurified use at 1/10000 - 1/20000.**
IP		1/30.
IHC-P		1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Flow Cyt		1/50 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

靶标

功能	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.
序列相似性	Contains 1 CSD (cold-shock) domain.
翻译后修饰	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.
细胞定位	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.

图片



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

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

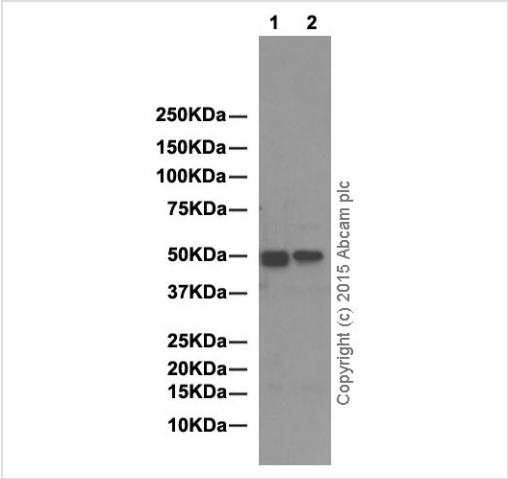
Lane 1 : NIH/3T3 whole cell lysate
Lane 2 : Raw264.7 whole cell lysate

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)

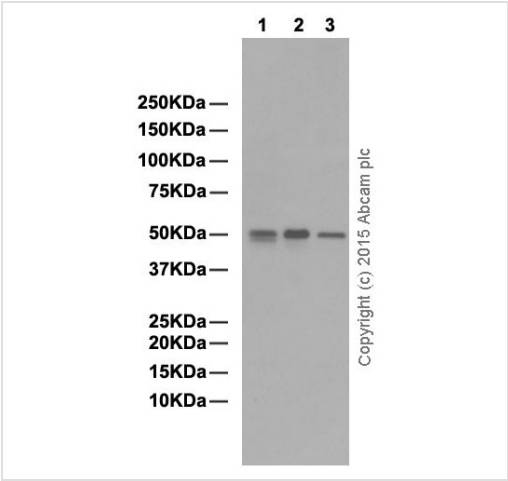
Lane 1 : C6 whole cell lysate
Lane 2 : PC-12 whole cell lysate

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)

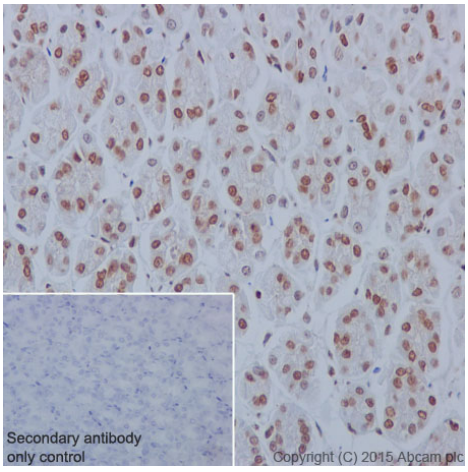
Lane 1 : HeLa whole cell lysate
Lane 2 : SW480 whole cell lysate
Lane 3 : A549 whole cell lysate

Lysates/proteins at 10 µg per lane.

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

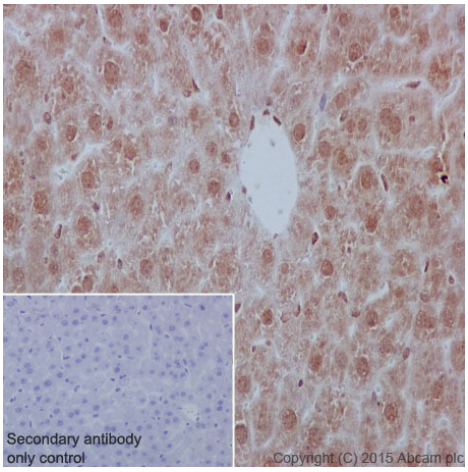
Predicted band size: 36 kDa
Observed band size: 50 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



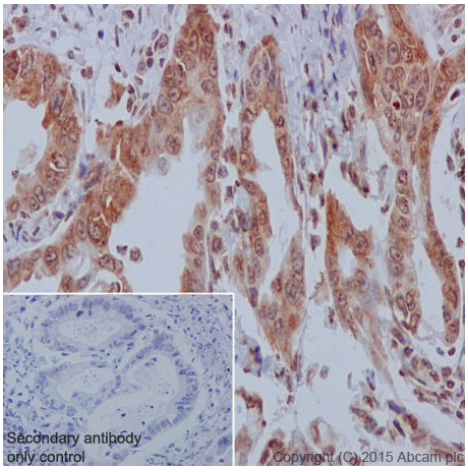
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

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.



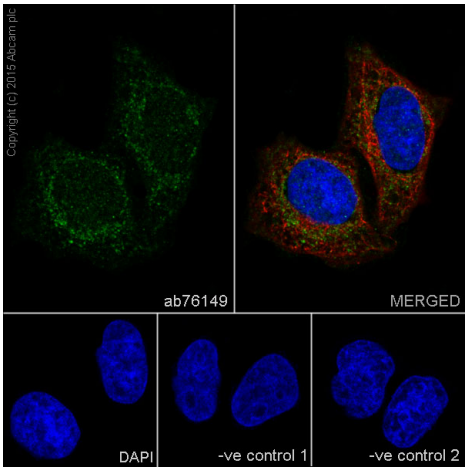
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

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.

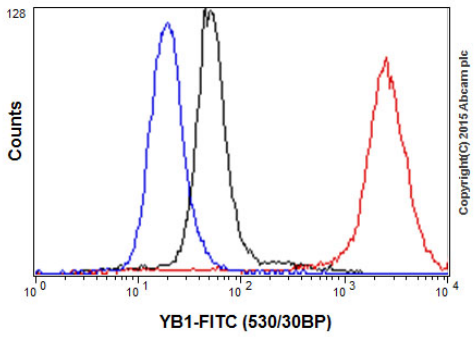


Immunocytochemistry/ Immunofluorescence - Anti-YB1 antibody [EP2708Y] (ab76149)

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.

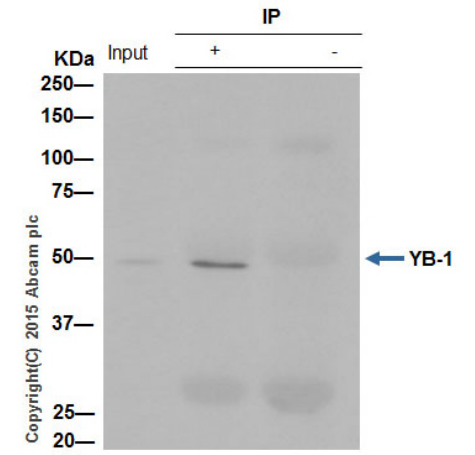
Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

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



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

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)

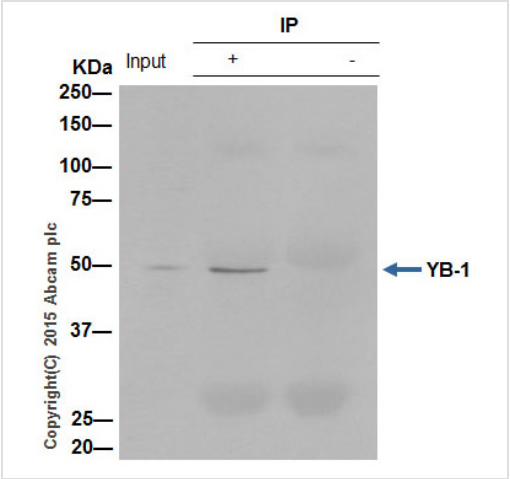
Lane 2 (+): ab76149 + MCF-7 whole cell lysate.

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)

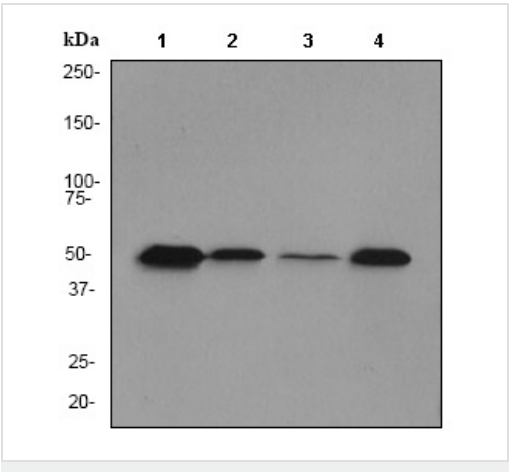
Lane 2 (+): ab76149 + HeLa whole cell lysate.

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)

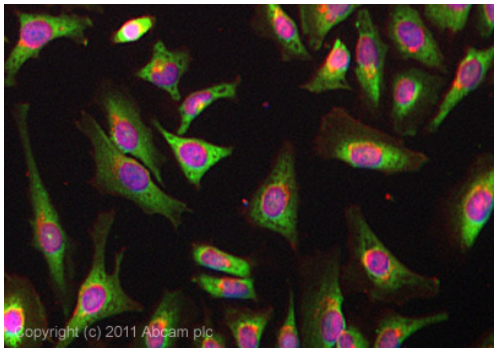
All lanes : Anti-YB1 antibody [EP2708Y] (ab76149) at 1/200000 dilution (unpurified)

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.

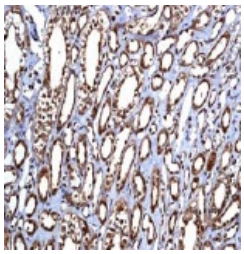
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/1000 dilution

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



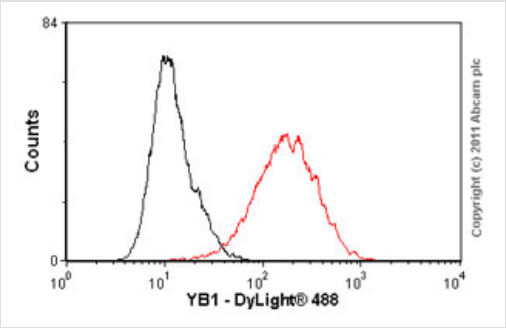
Immunocytochemistry/ Immunofluorescence - Anti-YB1 antibody [EP2708Y] (ab76149)

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.



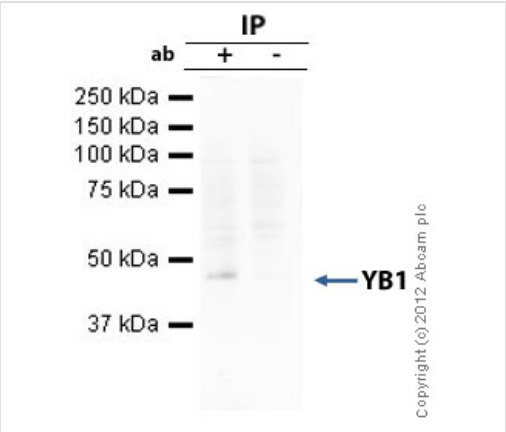
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YB1 antibody [EP2708Y] (ab76149)

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



Flow Cytometry - 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.

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