abcam

Product datasheet

Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] ab290734

Recombinant RabMAb

1 References 9 Images

Overview

Product name Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69]

Description Rabbit monoclonal [EPR26183-69] to YTHDF1 + YTHDF3 + YTHDF2

Host species Rabbit

Specificity Please note that this antibody does not react with Rat species for ICC and Flow Cyt (intra)

applications.

Tested applications Suitable for: WB, IP, Flow Cyt (Intra), ICC/IF

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa (human cervix adenocarcinoma epithelial cell) and 293T (human embryonic kidney

epithelial cell) whole cell lysates. NIH/3T3 (mouse embryonic fibroblast) whole cell lysate. PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate. ICC/IF: HeLa cells, NIH/3T3 cells. Flow

Cyt (intra): HeLa cells, NIH/3T3 cells. IP: HeLa and NIH/3T3 whole cell lysate

General notesThis 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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

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Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR26183-69

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab290734 in the following tested applications.

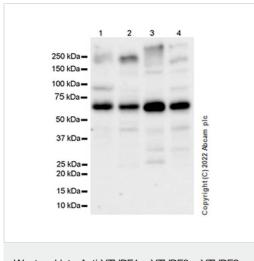
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 61 kDa (predicted molecular weight: 61 kDa).
IP		1/30.
Flow Cyt (Intra)		1/500.
ICC/IF		1/50.

Application notes

Is unsuitable for IHC-P.

Images



Western blot - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)

All lanes : Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 2: 293T (human embryonic kidney epithelial cell) whole cell lysate.

Lane 3: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate.Lane 4: PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate.

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 61 kDa Observed band size: 61 kDa Exposure time: 70 seconds

Blocking / Diluent buffer and concentration:5% NFDM/TBST.

Lysates should be made freshly and used in WB immediately to minimize protein degradation.

All lanes : Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734) at 1/1000 dilution

Lane 1 : His-tagged human YTHDF1 recombinant protein 10ng
Lane 2 : His-tagged human YTHDF2 recombinant protein 10ng

Lane 3: His-tagged human YTHDF3 recombinant protein 10ng

Secondary

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

dilution

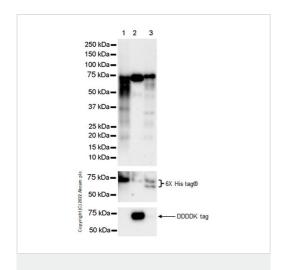
Predicted band size: 61 kDa **Observed band size:** 61 kDa

Exposure time: 70 seconds

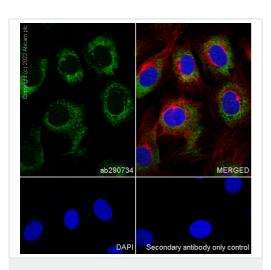
Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeilized NIH/3T3 (mouse embryonic fibroblast) cells lebelling YTHDF1 + YTHDF2 + YTHDF3 with ab290734 at 1/50 (10.04 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed antibody at 1/1000 (2ug/mL) dilution (Green). Confocal image showing cytoplasmic staining in NIH/3T3 cell line is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

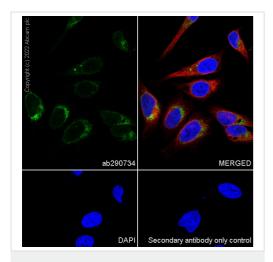
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/mL) dilution.



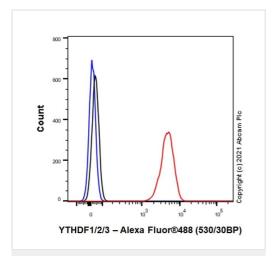
Western blot - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)



Immunocytochemistry/ Immunofluorescence - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)



Immunocytochemistry/ Immunofluorescence - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)

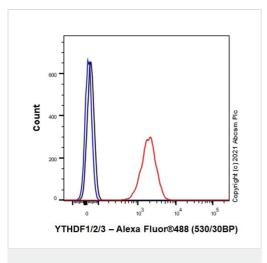


Flow Cytometry (Intracellular) - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)

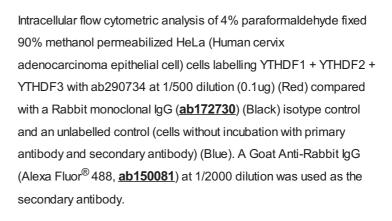
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeilized HeLa (human cervix adenocarcinoma epithelial cell) cells lebelling YTHDF1 + YTHDF2 + YTHDF3 with ab290734 at 1/50 (10.04 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2ug/mL) dilution (Green). Confocal image showing mostly cytoplasmic staining in Hela cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

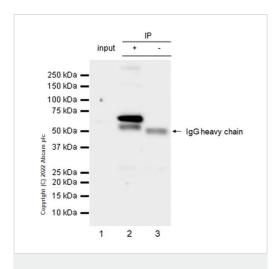
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/mL) dilution.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling YTHDF1 + YTHDF2 + YTHDF3 with ab290734 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)





Immunoprecipitation - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)

YTHDF1 + YTHDF2 + YTHDF3 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 ug with ab290734 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab290734 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

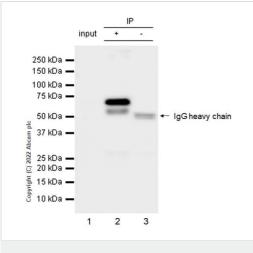
Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 ug

Lane 2: ab290734 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab290734 in NIH/3T3 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds



Immunoprecipitation - Anti-YTHDF1 + YTHDF3 + YTHDF2 antibody [EPR26183-69] (ab290734)

YTHDF1 + YTHDF2 + YTHDF3 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 ug ab290734 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using anti YTHDF1 + YTHDF2 + YTHDF3 antibody (ab290734) at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 μg

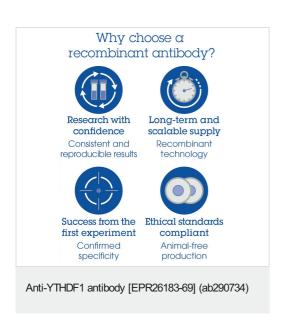
Lane 2: anti YTHDF1 + YTHDF2 + YTHDF3 antibody (ab290734)

IP in HeLa whole cell lysate

 $\label{lambda} \textbf{Lane 3:} \ \ \text{Rabbit monoclonal lgG } (\underline{\textbf{ab172730}}) \ \ \text{instead of ab290734}$ in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds



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