

Product datasheet

Anti-YTHDF3 antibody [EPR21912-3] ab220161

KO **VALIDATED** Recombinant **RabMAb**

★★★★★ 2 Abreviews 3 References 11 Images

Overview

Product name	Anti-YTHDF3 antibody [EPR21912-3]
Description	Rabbit monoclonal [EPR21912-3] to YTHDF3
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt, IP, WB, IHC-Fr, IHC-P Unsuitable for: ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment within Human YTHDF3 aa 350 to the C-terminus. The exact sequence is proprietary. Database link: Q7Z739
Positive control	WB: Wild-type mESC whole cell lysate. GST-tagged human YTHDF3 recombinant protein. Fresh HT-1080, HeLa, NIH/3T3, PC-12 whole cell lysate. IHC-P: Mouse and rat cerebrum tissue. Human colon tissue. IHC-Fr: Mouse and rat cerebrum tissue. Flow Cyt: HeLa cells. IP: HeLa whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.</p> <p>Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.</p> <p>We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise[™] guarantee.</p> <p>In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.</p>

We are also updating the applications & species that this product has been “predicted to work with,” however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21912-3
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab220161** 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		1/60.
IP		1/30.
WB	★★★★★	1/1000. Detects a band of approximately 73 kDa (predicted molecular weight: 64 kDa).
IHC-Fr		1/100.
IHC-P	★★★★★	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Use at 1/500 (mouse, rat) and 1/100 (human) dilution.

Application notes Is unsuitable for ICC/IF.

Target

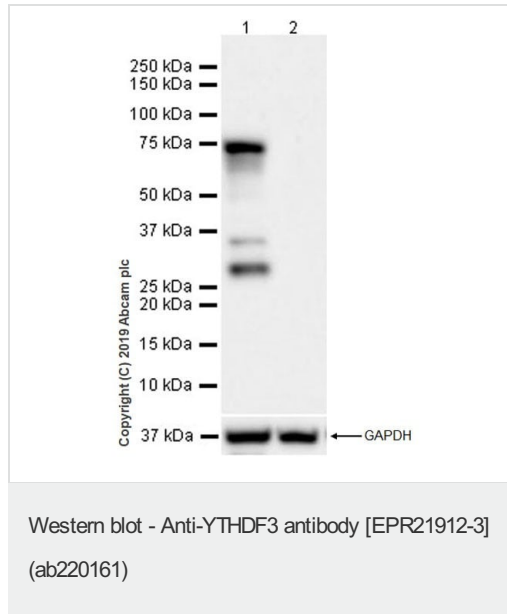
Function Specifically recognizes and binds N6-methyladenosine (m6A)-containing RNAs. M6A is a

modification present at internal sites of mRNAs and some non-coding RNAs and plays a role in the efficiency of mRNA splicing, processing and stability.

Sequence similarities

Contains 1 YTH domain.

Images



All lanes : Anti-YTHDF3 antibody [EPR21912-3] (ab220161) at 1/1000 dilution

Lane 1 : Wild-type mESC (mouse embryo stem cell) whole cell lysate

Lane 2 : YTHDF3 knockout mESC whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 64 kDa

Observed band size: 73 kDa

[why is the actual band size different from the predicted?](#)

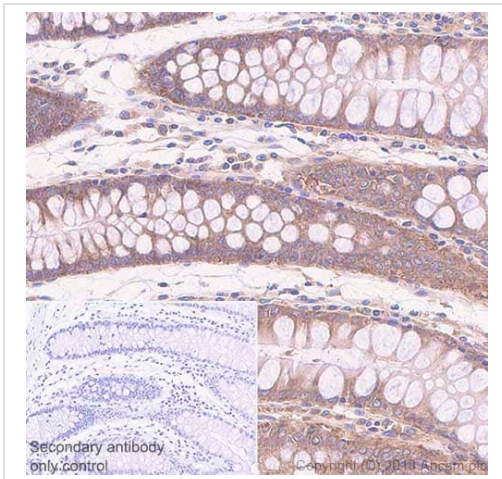
Exposure time: 59 seconds

The wild-type and YTHDF3 knockout cell lysates were kindly provided by an anonymous collaborator.

ab220161 was shown to specifically react with YTHDF3 in wild-type mESC cells as signal was lost in YTHDF3 knockout cells. Wild-type and YTHDF3 knockout samples were subjected to SDS-PAGE. ab220161 and [ab181602](#) (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated ([ab97051](#)) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging.

The blot was developed on a BIO-RAD[®] ChemiDoc[™] MP instrument using the ECL technique.

Blocking/Dilution buffer: 5% NFDm/TBST.

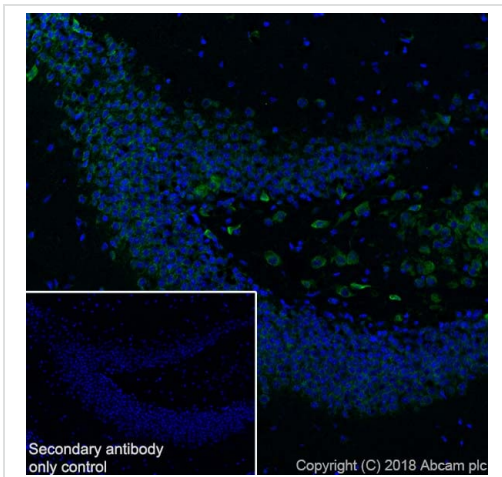


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling YTHDF3 with ab220161 at 1/100 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on human colon (PMID:29103884, 28250115) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).

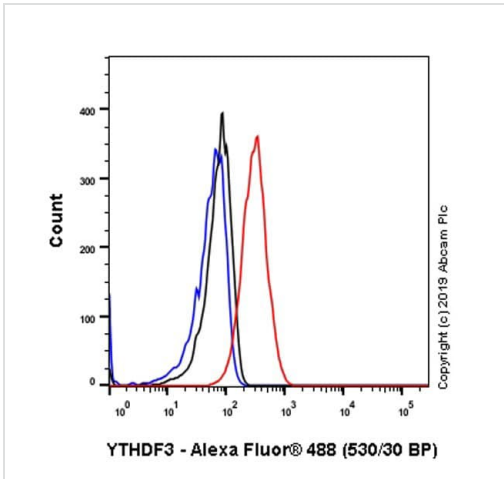


Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebrum tissue labeling YTHDF3 with ab220161 at 1/100 dilution (green), followed by [ab150077](#) AlexaFluor[®]488 Goat anti-Rabbit secondary at a 1/1,000 dilution. Cytoplasmic and nuclear staining in mouse cerebrum (PMID:29103884, 28250115) is observed. Counterstained with DAPI (blue).

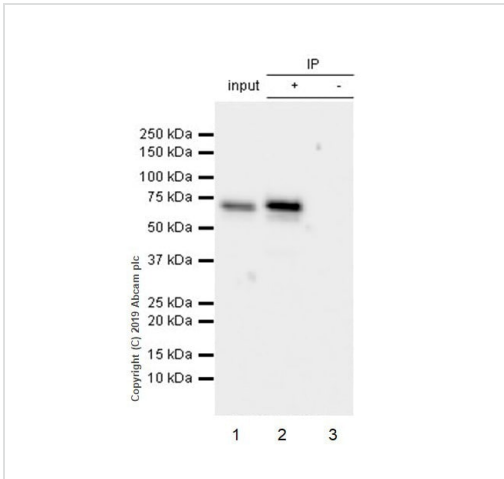
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab150077](#) AlexaFluor[®]488 Goat anti-Rabbit used at a 1/1,000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).



Flow Cytometry - Anti-YTHDF3 antibody
[EPR21912-3] (ab220161)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling YTHDF3 with ab220161 at 1/60 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-YTHDF3 antibody
[EPR21912-3] (ab220161)

YTHDF3 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with ab220161 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab220161 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).

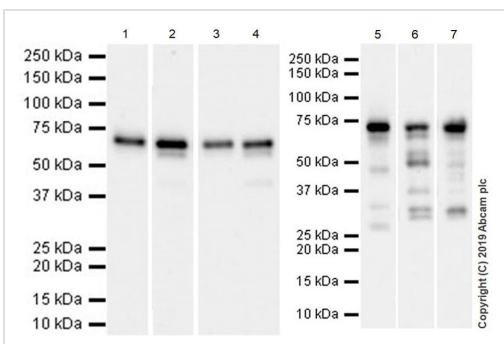
Lane 2: ab220161 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab220161 in HeLa whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 15 seconds.

Lysate were made freshly and used in IP test immediately to minimize protein degradation. Incubation time was 2h.



Western blot - Anti-YTHDF3 antibody [EPR21912-3]
(ab220161)

All lanes : Anti-YTHDF3 antibody [EPR21912-3] (ab220161) at 1/1000 dilution

Lane 1 : HT-1080 (human fibrosarcoma epithelial cell) whole cell lysate (fresh lysate)

Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate (fresh lysate)

Lane 3 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate (fresh lysate)

Lane 4 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate (fresh lysate)

Lane 5 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 6 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lane 7 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

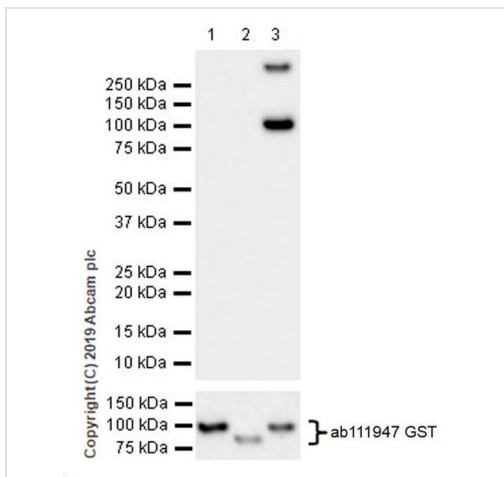
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 64 kDa

Lysate should be made freshly and used in WB immediately to minimize protein degradation (lane1-4). Lane 5-7 are the lysates from same cell lines but have experienced freeze-thaw cycles.

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure times: Lanes 1-4: 37 secs; Lanes 5-7: 92 secs.



Western blot - Anti-YTHDF3 antibody [EPR21912-3] ([ab220161](#))

All lanes : Anti-YTHDF3 antibody [EPR21912-3] ([ab220161](#)) at 1/5000 dilution

Lane 1 : GST-tagged human YTHDF1 recombinant protein 20 ng

Lane 2 : GST-tagged human YTHDF2 recombinant protein 20 ng

Lane 3 : GST-tagged human YTHDF3 recombinant protein 20 ng

Secondary

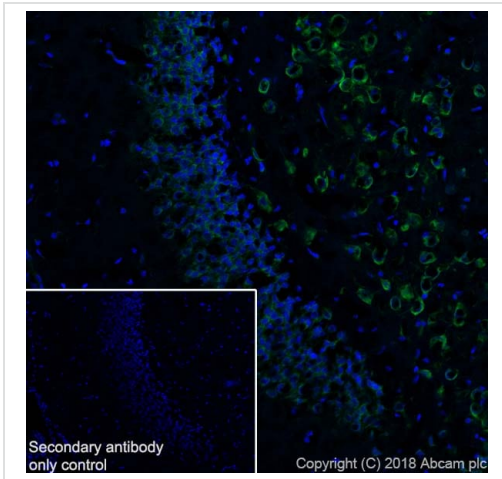
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 64 kDa

Exposure time: 10 seconds

The YTHDF recombinant proteins were kindly provided by an anonymous collaborator.

Blocking/Dilution buffer: 5% NFDm/TBST.

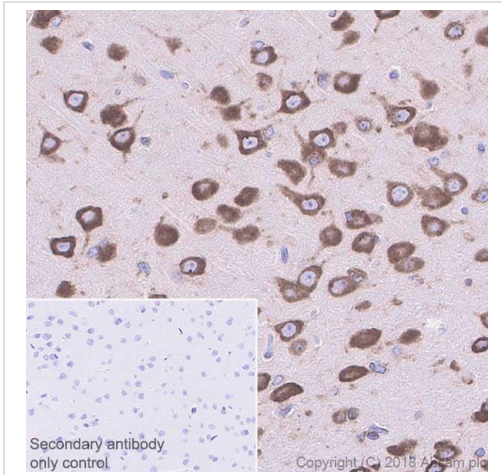


Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat cerebrum tissue labeling YTHDF3 with ab220161 at 1/100 dilution (green), followed by [ab150077](#) AlexaFluor[®]488 Goat anti-Rabbit secondary at a 1/1,000 dilution. Cytoplasmic and nuclear staining in rat cerebrum (PMID:29103884, 28250115) is observed. Counterstained with DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab150077](#) AlexaFluor[®]488 Goat anti-Rabbit used at a 1/1,000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).

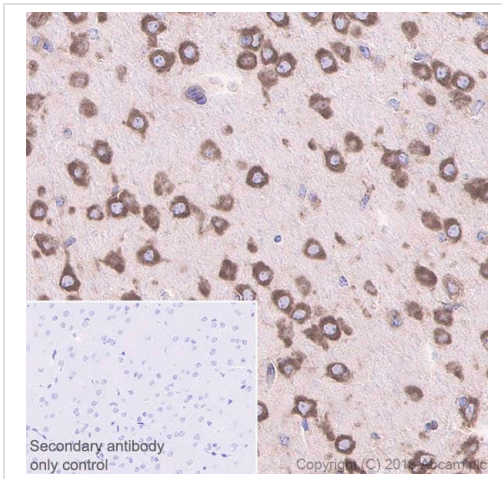


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue labeling YTHDF3 with ab220161 at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on neurons of rat cerebrum (PMID:29103884, 28250115) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling YTHDF3 with ab220161 at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on neurons of mouse cerebrum (PMID:29103884, 28250115) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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