abcam

ab220161

Anti-YTHDF3 antibody [EPR21912-3]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR21912-3
Purification technique	Affinity purification Protein A
Concentration	0.526 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Not recommended

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

IHC-Fr

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Species	Rat
Dilution info	1/100
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/60
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	Use at 1/500 (mouse, rat) and 1/100 (human) dilution. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	Use at 1/500 (mouse, rat) and 1/100 (human) dilution.

	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	-
Notes	Use at 1/500 (mouse, rat) and 1/100 (human) dilution. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information YTHDF3

Function	Specifically recognizes and binds N6-methyladenosine (m6A)-containing RNAs, and regulates their stability (PubMed:28106072, PubMed:28106076, PubMed:28281539, PubMed:32492408). M6A is a modification present at internal sites of mRNAs and some non-coding RNAs and plays a role in mRNA stability and processing (PubMed:22575960, PubMed:24284625, PubMed:28106072, PubMed:28281539, PubMed:32492408). Acts as a regulator of mRNA stability by promoting degradation of m6A-containing mRNAs via interaction with the CCR4-NOT complex or PAN3 (PubMed:32492408). The YTHDF paralogs (YTHDF1, YTHDF2 and YTHDF3) share m6A-containing mRNAs targets and act redundantly to mediate mRNA degradation and cellular differentiation (PubMed:28106072, PubMed:28106076, PubMed:32492408). Acts as a negative regulator of type I interferon response by down-regulating interferon-stimulated genes (ISGs) expression: acts by binding to FOXO3 mRNAs (By similarity). Binds to FOXO3 mRNAs independently of METTL3-mediated m6A modification (By similarity). Can also act as a regulator of mRNA stability in cooperation with YTHDF2 by binding to m6A-containing mRNA and promoting their degradation (PubMed:28106072). Recognizes and binds m6A-containing circular RNAs (circRNAs); circRNAs are generated through back-splicing of pre-mRNAs, a non-canonical splicing process promoted by dsRNA structures across circularizing exons (PubMed:28281539). Promotes formation of phase-separated membraneless compartments, such as P-bodies or stress granules, by undergoing liquid-liquid phase separation upon binding to mRNAs containing multiple m6A-modified residues: polymethylated mRNAs act as a multivalent scaffold for the binding of YTHDF proteins, juxtaposing their disordered regions and thereby leading to phase separation (PubMed:31292544, PubMed:31388144, PubMed:32451507). The resulting mRNA-YTHDF complexes then partition into different endogenous phase-separated membraneless compartments, such as P-bodies, stress granules or neuronal RNA granules (PubMed:31292544). May also recognize and bind N1-methyladenosine (m1A)-containing mRNAs: inhibits trophoblast invasion by binding to m1A-methylated transcripts of IGF1R, promoting their degradation (PubMed:32194978). Has some antiviral activity against HIV-1 virus: incorporated into HIV-1 particles in a nucleocapsid-dependent manner and reduces viral infectivity in the next cycle of infection (PubMed:32053707). May interfere with this early step of the viral life cycle by binding to N6-methyladenosine (m6A) modified sites on the HIV-1 RNA genome (PubMed:32053707).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The YTH domain family protein 3 (YTHDF3) is an m6A reader protein known also as YTH3 or YTHDF2-like protein 3. Its molecular weight is approximately 64 kDa. YTHDF3 plays a role in the recognition of N6-methyladenosine (m6A)-modified RNA an important post-transcriptional modification. It commonly expresses in various tissues including the brain liver and kidney indicating its involvement in multiple cellular processes. YTHDF3 binds to m6A-modified mRNA influencing the stability and translation of these molecules and interacts with other members of the YTH family.
Biological function summary	YTHDF3 facilitates the process of mRNA metabolism by participating in the regulation of mRNA translation and degradation. It functions as part of a dynamic complex with YTHDF1 and YTHDF2 coordinating the fate of m6A-modified mRNAs.

	YTHDF3 supports mRNA decay in synergy with YTHDF2 and enhances translation efficiency in cooperation with YTHDF1. This involvement in regulating gene expression at the post-transcriptional level equips cells to respond rapidly to various stimuli aligning gene expression with cellular needs.
Pathways	YTHDF3 significantly impacts RNA processing and metabolism pathways. YTHDF3 together with YTHDF1 and YTHDF2 modulates the m6A-mediated mRNA decay pathway. It holds functional significance in the cellular response to stress and adaptations in energy metabolism. YTHDF3 interacts closely with proteins such as IGF2BP1 alongside the YTHDF proteins which are critical in maintaining RNA stability and translation thereby illustrating its role across significant cellular pathways.
Associated diseases and disorders	YTHDF3 has implications in cancer and neurodegenerative diseases. High YTHDF3 expression associates with various cancer types including gliomas and breast cancer where it can influence tumor growth and metastasis. In neurodegenerative diseases such as Alzheimer's disease YTHDF3 interacts with proteins like YTHDF1 and YTHDF2 affecting RNA metabolism in neurons contributing potentially to the pathology of these disorders. Through these interactions YTHDF3 could impact disease progression and outcomes making it a target of interest in therapeutic research.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

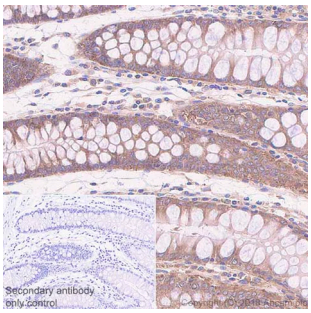
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

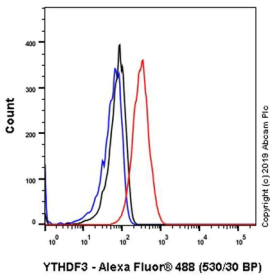
12 product images



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).

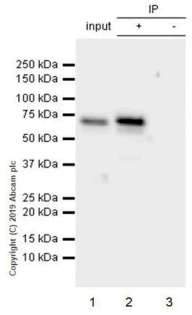
Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



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

YTHDF3 Flow Cytometry (Intracellular) staining using rabbit Anti-YTHDF3 antibody

Intracellular 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.

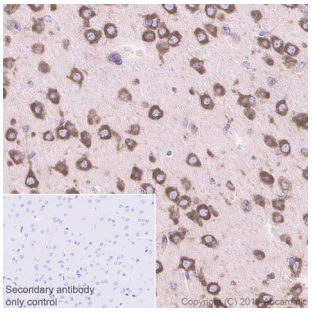
Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: 15 seconds.

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

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

Predicted band size: 64 kDa

Observed band size: 73 kDa

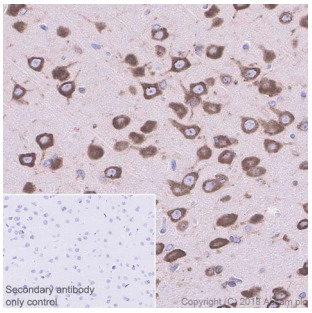


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

YTHDF3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-YTHDF3 antibody

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).

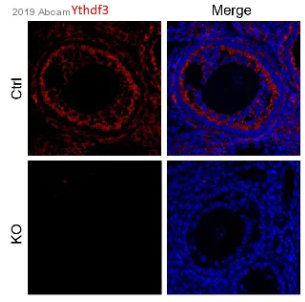


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

YTHDF3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-YTHDF3 antibody

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).

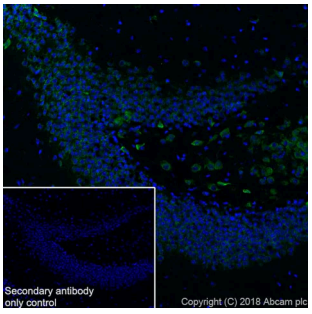
Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



This image is courtesy of a customer review submitted by Bin Shen

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

Immunohistochemistry analysis (Formalin/PFA-fixed paraffin-embedded sections) of paraformaldehyde-fixed mouse ovary tissue permeabilized with PBST (0.5% Triton X-100 in PBS). Stained with ab220161 at 1/200 dilution. Secondary antibody used was Alex Fluor 555 donkey anti-rabbit IgG at 1/500 dilution. Blocking was done with 5% BSA in PBST (0.1% tween in PBS) for 1 hour 30 minutes at 25°C. The sample was incubated with the primary antibody and 5% BSA in PBST (0.1% tween in PBS) for 12 hours at 4°C. Antigen retrieval method was heat mediated, Citrate antigen repair solution.



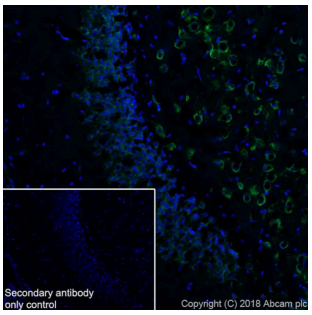
Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

YTHDF3 Immunohistochemistry (Frozen sections) staining using rabbit Anti-YTHDF3 antibody

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).

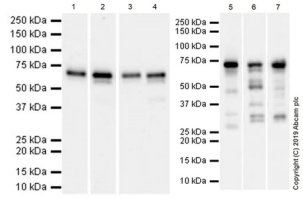


Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

YTHDF3 Immunohistochemistry (Frozen sections) staining using rabbit Anti-YTHDF3 antibody

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).



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

YTHDF3 Western blot staining using rabbit Anti-YTHDF3 antibody

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.

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

Lane 1:
HT-1080 (human fibrosarcoma epithelial cell) whole cell lysate (fresh lysate) at 20 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate (fresh lysate) at 20 µg

Lane 3:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate (fresh lysate) at 20 µg

Lane 4:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate (fresh lysate) at 20 µg

Lane 5:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

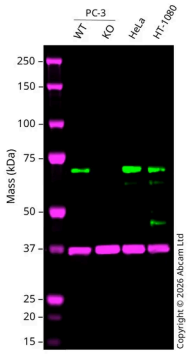
Lane 6:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 7:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

Secondary

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

Predicted band size: 64 kDa



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

YTHDF3 Western blot staining using rabbit Anti-YTHDF3 antibody

Western blot: Anti-YTHDF3 antibody [EPR21912-3] ab220161 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 70 kDa in Wild-type PC-3 cell lysates with no signal observed at this size in YTHDF3 knockout PC-3 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-YTHDF3 antibody [EPR21912-3] (ab220161) at 1/1000 dilution

Lane 1:
Wild-type PC-3 cell lysates at 20 µg

Lane 2:
Western blot - Human YTHDF3 knockout PC-3 cell line (ab290587) at 20 µg

Lane 3:
HeLa cell lysates at 20 µg

Lane 4:
HT-1080 cell lysates at 20 µg

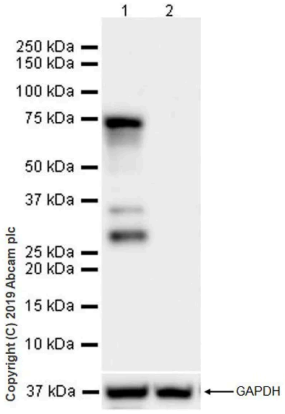
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 64 kDa

Observed band size: 70 kDa



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

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.

All lanes:

Western blot - Anti-YTHDF3 antibody [EPR21912-3] (ab220161) at 1/1000 dilution

Lane 1:
Wild-type mESC (mouse embryo stem cell) whole cell lysate at 20 µg

Lane 2:
YTHDF3 knockout mESC whole cell lysate at 20 µg

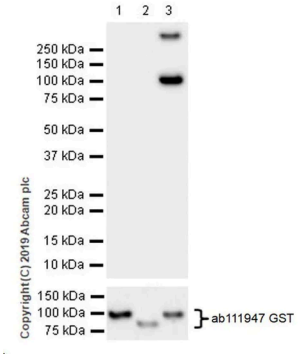
Secondary

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

Predicted band size: 64 kDa

Observed band size: 73 kDa

Exposure time: 59s



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

The YTHDF recombinant proteins were kindly provided by an anonymous collaborator.
Blocking/Dilution buffer: 5% NFDM/TBST.

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

Predicted band size: 64 kDa

Exposure time: 10s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.