abcam

Product datasheet

Anti-YTHDF3 antibody [EPR21912-3] ab220161

KO VALIDATED Recombinant RabMAb

3 Abreviews 17 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 (Intra), IP, WB, IHC-Fr, IHC-P **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
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 (intra): HeLa cells. IP: HeLa whole cell lysate.
General notes	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**.

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

The Abpromise guarantee 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 (Intra)		1/60.
IP		1/30.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 73 kDa (predicted molecular weight: 64 kDa).
IHC-Fr		1/100.
IHC-P	★★★★★ (1)	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.

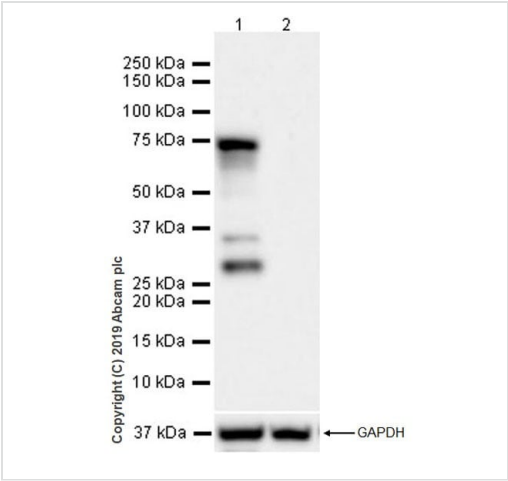
Application notes Is unsuitable for ICC/IF.

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



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

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

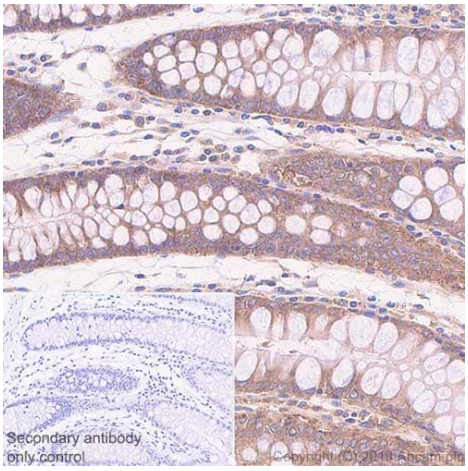
Exposure time: 59 seconds

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.

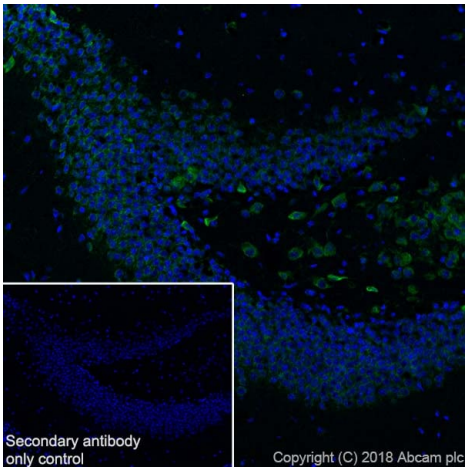


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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).

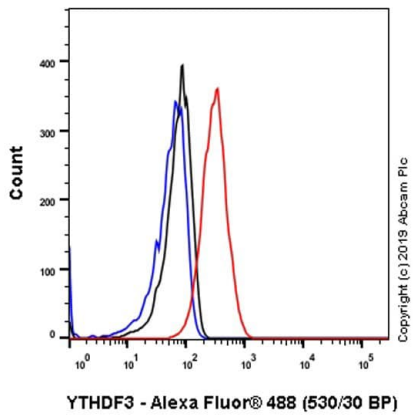


Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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).

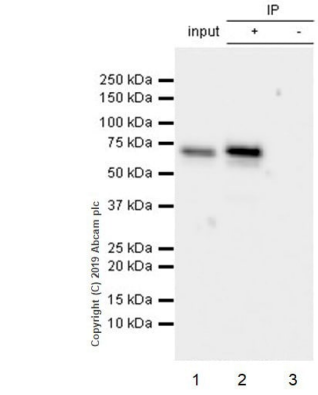
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit used at a 1/1,000 dilution.

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



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

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.



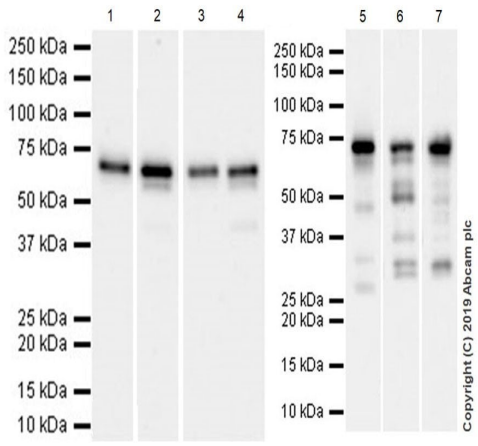
Immunoprecipitation - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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.



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.

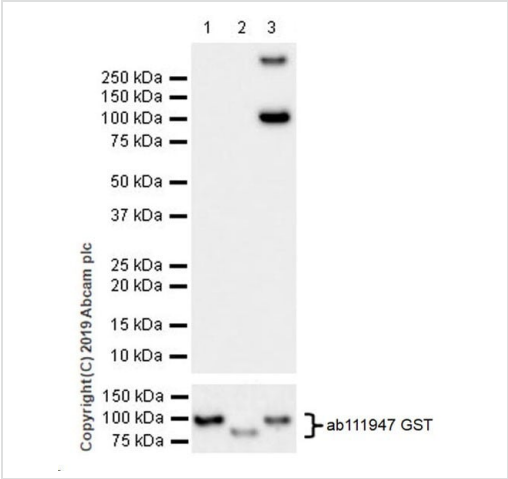
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

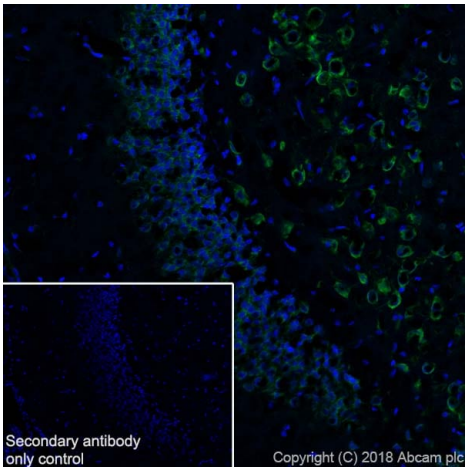
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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.

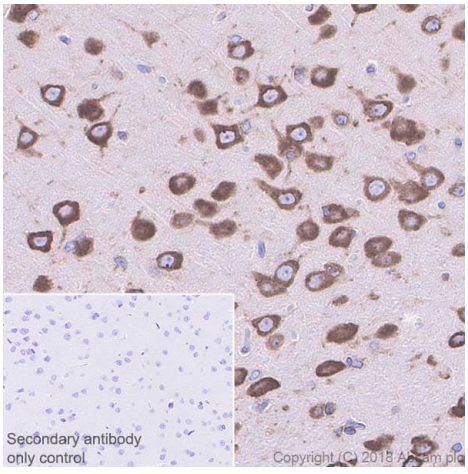


Immunohistochemistry (Frozen sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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).

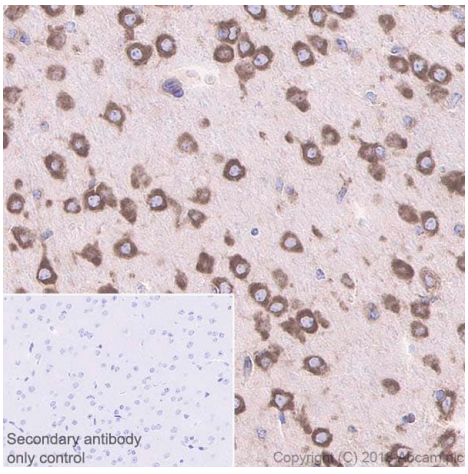


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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).

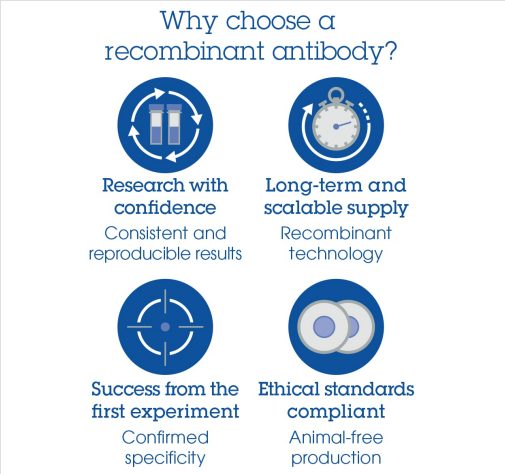


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF3 antibody [EPR21912-3] (ab220161)

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).



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

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