abcam

Product datasheet

Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free ab275037

Recombinant RabMAb

12 Images

Overview

Product name	Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free
Description	Rabbit monoclonal [EPR23544-19] to YTHDF2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, Flow Cyt (Intra), IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, LNCaP, MEF and PC-12 whole cell lysates; His-tagged human YTHDF2 recombinant protein. IHC-P: Human testis tissue; Mouse testis tissue; Rat testis tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa and NIH/3T3 cells. IP: HeLa and NIH/3T3 whole cell lysates.
General notes	ab275037 is the carrier-free version of **ab246514**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23544-19
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275037 in the following tested applications.

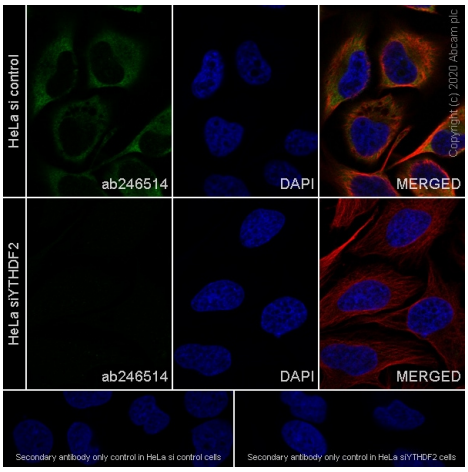
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 62 kDa (predicted molecular weight: 62 kDa).

Target

Sequence similarities	Contains 1 YTH domain.

Images

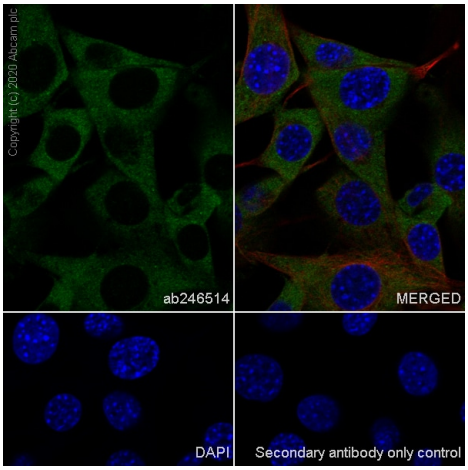


Immunocytochemistry/ Immunofluorescence - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial adenocarcinoma cell line) (siRNA control) and HeLa (siRNA YTHDF) cells labelling YTHDF2 with **ab246514** at 1/100 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HeLa cell line transfected with scramble siRNA and reduced staining in HeLa cell line transfected with siRNA YTHDF2. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

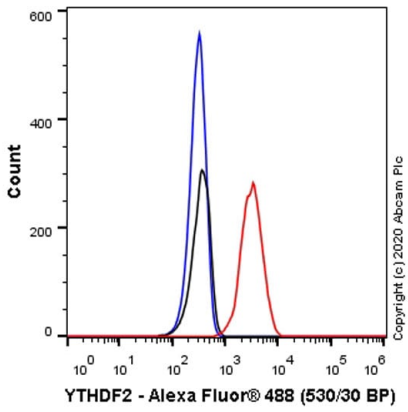


Immunocytochemistry/ Immunofluorescence - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilizedNIH/3T3 (Mouse embryo fibroblast cell line) cells labelling YTHDF2 with **ab246514** at 1/50 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in NIH/3T3 cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

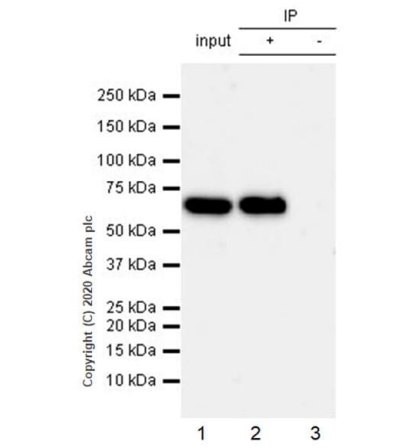


Flow Cytometry (Intracellular) - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling YTHDF2 with **ab246514** at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor®488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

YTHDF2 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast cell line) whole cell lysate with **ab246514** at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab246514** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

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

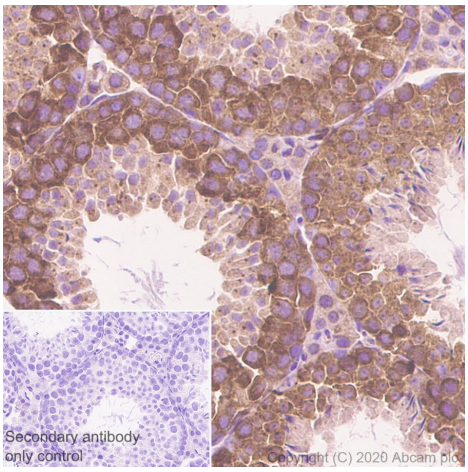
Lane 2: **ab246514** IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab246514** in NIH/3T3 whole cell lysate

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

Exposure time: 84 seconds.

Lysates were made freshly and used in IP test immediately to minimize protein degradation.



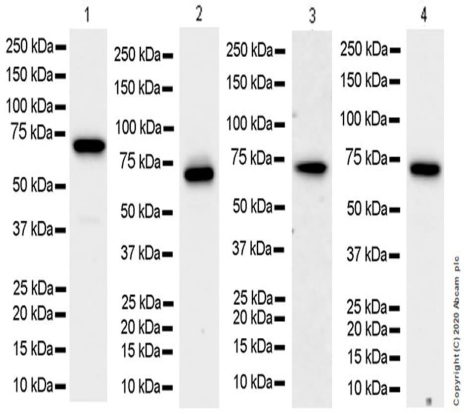
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling YTHDF2 with **ab246514** at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Cytoplasmic staining in mouse testis (PMID: 30903744, PMID: 29103884). The section was incubated with **ab246514** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

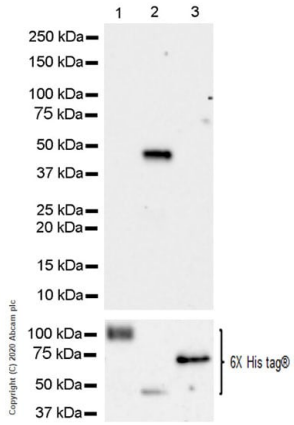
All lanes : Anti-YTHDF2 antibody [EPR23544-19] (**ab246514**) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : LNCaP (human prostate carcinoma epithelial cell) whole cell lysate
Lane 3 : MEF (mouse embryonic fibroblast (immortalized)) 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), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 62 kDa
Observed band size: 62 kDa



Western blot - Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-2: 37 seconds; Lane 3: 180 seconds; Lane 4:37 seconds.

The molecular mass observed is consistent with the literature (PMID: 28104805).

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

All lanes : Anti-YTHDF2 antibody [EPR23544-19] (**ab246514**) at 1/5000 dilution

Lane 1 : LIF-His-tagged human YTHDF1 recombinant protein, 10 ng
Lane 2 : His-tagged human YTHDF2 recombinant protein, 10 ng
Lane 3 : His-tagged human YTHDF3 recombinant protein, 10 ng

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

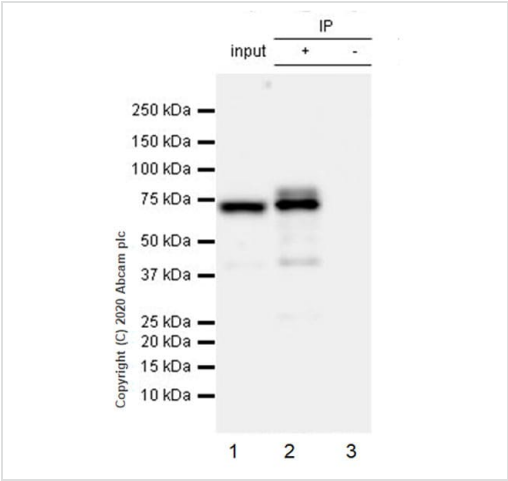
Predicted band size: 62 kDa
Observed band size: 62 kDa

This data was developed using **ab246514**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 59 seconds.

These rec proteins were made in house.
LIF-His-tagged human YTHDF1 recombinant protein was expressed from a mammalian - HEK-293 expression system.
His-tagged human YTHDF2 recombinant protein is extracts of E.coli expressing YTHDF2.
His-tagged human YTHDF3 recombinant protein is extracts of E.coli expressing YTHDF3.



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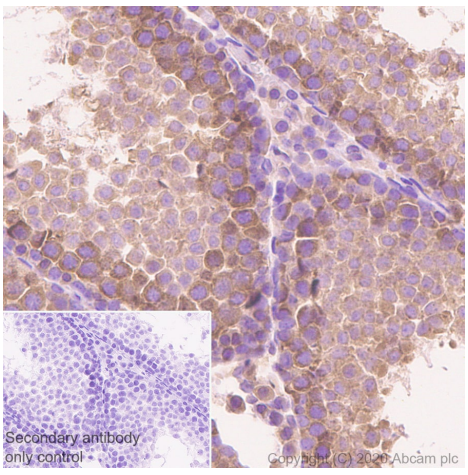
Lane 1: HeLa whole cell lysate 10 ug

Lane 2: **ab246514** IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab246514** in HeLa whole cell lysate

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

Exposure time: 10 seconds.



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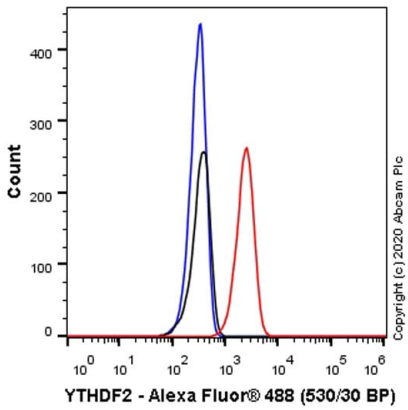

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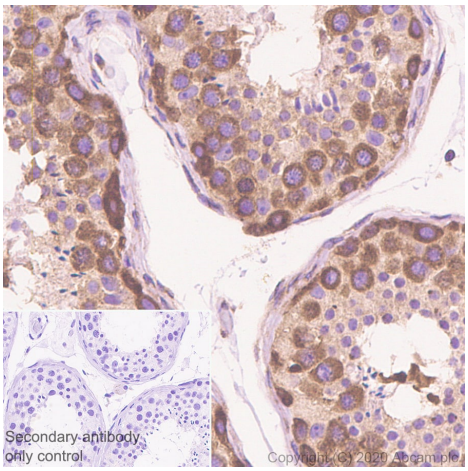

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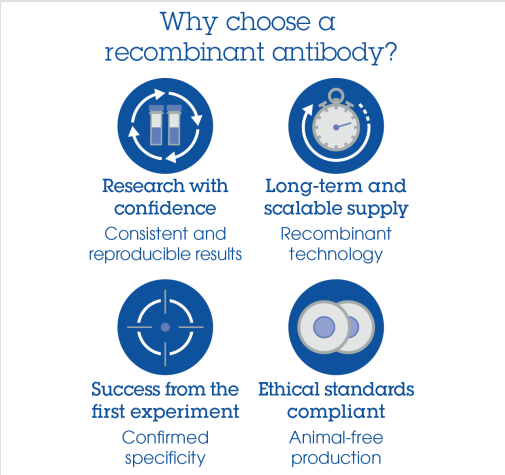

Anti-YTHDF2 antibody [EPR23544-19] - BSA and Azide free (ab275037)

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