# abcam

# Product datasheet

# Anti-YTHDF2 antibody [EPR20318] ab220163





★★★★★ 3 Abreviews 43 References 6 Images

Overview

**Product name** Anti-YTHDF2 antibody [EPR20318]

**Description** Rabbit monoclonal [EPR20318] to YTHDF2

**Host species** Rabbit

**Tested applications** Suitable for: WB, IP

Unsuitable for: Flow Cyt,ICC/IF or IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Wild-type mESC, HEK-293T, HT-1080 treated with 10µM MG-132 for 4 hours, K562, HEK-

293 and HeLa whole cell lysate. Human brain, mouse and rat testis tissue lysate; GST-tagged

human YTHDF2 recombinant protein. IP: HepG2 cells

**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** 

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

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR20318

**Isotype** IgG

## **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab220163 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	<b>★★★★★ (2)</b>	1/1000. Predicted molecular weight: 62 kDa.
IP		1/30.

**Application notes** 

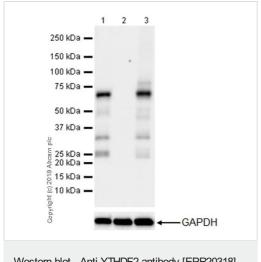
Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

# **Target**

Sequence similarities

Contains 1 YTH domain.

#### **Images**



Western blot - Anti-YTHDF2 antibody [EPR20318] (ab220163)

**All lanes :** Anti-YTHDF2 antibody [EPR20318] (ab220163) at 1/1000 dilution

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

Lane 2: YTHDF2 knockout mESC whole cell lysate

Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

# Secondary

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

Predicted band size: 62 kDa

Exposure time: 48 seconds

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

The wild-type and YTHDF2 knockout cell lysates were kindly

provided by an anonymous collaborator.

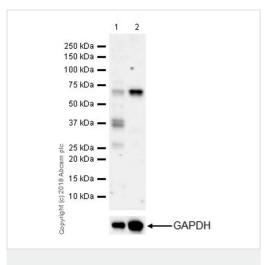
ab220163 was shown to specifically react with YTHDF2 in wild-type mESC cells as signal was lost in YTHDF2 knockout cells. Wild-type and YTHDF2 knockout samples were subjected to SDS-PAGE. ab220163 and <u>ab181602</u> (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP instrument using the ECL technique.

Lysate kept at -80°C Lysate made same day as IP 250 kDa 150 kDa -100 kDa -100 kDa -75 kDa -75 kDa -50 kDa • - IgG heavy chair 50 kDa -2 37 kDa **−** 25 kDa -20 kDa -© 15 kDa -15 kDa 🕳 10 kDa = 10 kDa -1 2 1 2 3 (A) (B)

Immunoprecipitation - Anti-YTHDF2 antibody [EPR20318] (ab220163)

ab220163 Immunoprecipitating YTHDF2 in human HepG2 whole cell lysate. 10 μg of cell lysate was incubated with primary antibody (1/30). For western blotting ab220163 (1/1000) was used to confirm successful immunoprecipitation. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at (1/5000). For lane 3 Rabbit monoclonal IgG (ab172730) was used instead of ab220163.

In panel A, the lysate was stored at -80°C prior to the IP and incubation with the primary antibody was carried out overnight. In panel B, lysate was freshly made and used in the IP test immediately to minimize protein degradation. The incubation time was also shortened from overnight to 2h.



Western blot - Anti-YTHDF2 antibody [EPR20318] (ab220163)

**All lanes :** Anti-YTHDF2 antibody [EPR20318] (ab220163) at 1/5000 dilution

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

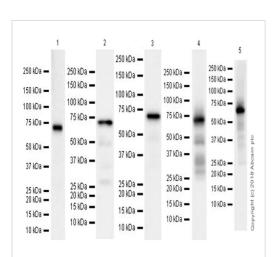
**Lane 2**: HT-1080 treated with  $10\mu M$  MG-132 (<u>ab141003</u>) for 4 hours, whole cell lysate at  $10\mu g$ 

#### **Secondary**

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

Predicted band size: 62 kDa

Exposure time: 103 seconds



Western blot - Anti-YTHDF2 antibody [EPR20318] (ab220163)

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

**All lanes :** Anti-YTHDF2 antibody [EPR20318] (ab220163) at 1/1000 dilution

Lane 1: Human brain tissue lysate

Lane 2: Mouse testis tissue lysate

Lane 3: Rat testis tissue lysate

Lane 4: HEK-293 (human embryonic kidney epithelial cell) whole cell lysate

**Lane 5**: K562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate

Lysates/proteins at 10 µg per lane.

## Secondary

**Lane 1 :** VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

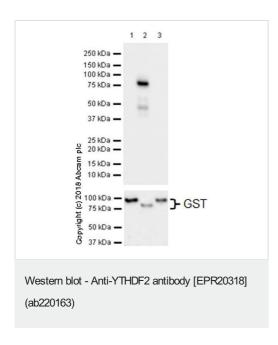
**Lanes 2-5 :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at 1/100000 dilution

Predicted band size: 62 kDa

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

Exposure time: Lane 1: 3 minutes; Lane 2: 92 seconds; Lane 3: 37

seconds; Lane 4: 8 seconds; Lane 5: 15 seconds.



**All lanes :** Anti-YTHDF2 antibody [EPR20318] (ab220163) at 1/1000 dilution

Lane 1: GST-tagged human YTHDF1 recombinant protein, 20ng
Lane 2: GST-tagged human YTHDF2 recombinant protein, 20ng
Lane 3: GST-tagged human YTHDF3 recombinant protein, 20ng

#### **Secondary**

All lanes : Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at

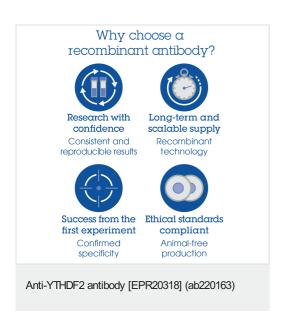
1/100000 dilution

Predicted band size: 62 kDa

Exposure time: 3 seconds

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

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



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

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