abcam

Product datasheet

Anti-METTL3 antibody [EPR18810] ab195352





重组 RabMAb

★★★★★ 8 Abreviews 237 References 19 图像

概述

产品名称 Anti-METTL3抗体[EPR18810]

描述 兔单克隆抗体[EPR18810] to METTL3

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

种属反应性 与反应: Mouse, Rat, Human

预测可用于: Sheep, Goat, Horse, Guinea pig, Cow, Cat, Dog, Pig, Non human primates

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Raji, HeLa, HEK-293, Jurkat, NCCIT, F9, Neuro-2a, LLC1, C6, RAW 264.7, PC-12 and

> NIH\3T3 cell lysates; human thymus lysate, mouse brain, spleen and heart lysates; rat brain lysate. IHC-P: Human bladder cancer, mouse testis and rat testis tissues, mouse skin, rat skin. ICC/IF:

HCT116 and HeLa, NIH/3T3 cells. IP: HeLa cell lysate.

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

纯度 Protein A purified

单**克隆** 克隆

克隆编号 EPR18810

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab195352于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (4)	1/1000. Detects a band of approximately 64 kDa (predicted molecular weight: 64 kDa). Milk recommended as blocking agent.
IHC-P	★★★★☆ (1)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	*** <u>*</u>	1/1000.
IP		1/50.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

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功能 N6-methyltransferase that methylates adenosine residues of some mRNAs. N6-methyladenosine

(m6A), which is present at internal sites of some mRNAs, may play a role in the efficiency of

mRNA splicing, transport or translation.

组织特异性 Widely expressed at low level. Expressed in spleen, thymus, prostate, testis, ovary, small

intestine, colon and peripheral blood leukocytes.

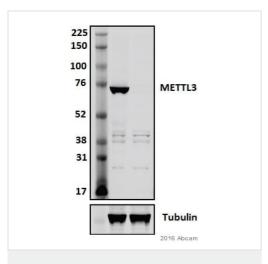
序列相似性 Belongs to the MT-A70-like family.

翻译后修饰 Phosphorylated upon DNA damage, probably by ATM or ATR.

细胞定位 Nucleus speckle. Colocalizes with speckles in interphase nuclei. Suggesting that it may be

associated with nuclear pre-mRNA splicing components.

图片



Western blot - Anti-METTL3 antibody [EPR18810] (ab195352)

This image is courtesy of an Abreview by Pedro Batista.

All lanes : Anti-METTL3 antibody [EPR18810] (ab195352) at 1/1000 dilution

Lane 1: Mouse embryonic stem cells

Lane 2: Mouse embryonic stem cells (METTL3 Knock-out)

Lysates/proteins at 15 µg per lane.

Secondary

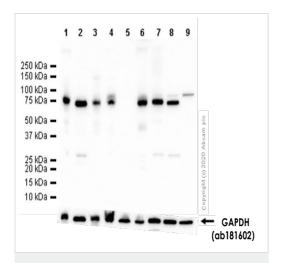
All lanes: Goat anti-Rabbit Polyclonal at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 64 kDa

Exposure time: 5 minutes

Blocking step: 5% Milk for 30 minutes at 25°C.



Western blot - Anti-METTL3 antibody [EPR18810] (ab195352)

All lanes : Anti-METTL3 antibody [EPR18810] (ab195352) at 1/1000 dilution

Lane 1 : Mouse brain lysate prepared in 1%SDS Hot lysis method at 20 μg

Lane 2: Mouse brain lysate prepared in RIPA lysis method at 20 mg/ml

Lane 3 : Mouse heart lysate prepared in 1%SDS Hot lysis method at 20 μg

Lane 4 : Mouse heart lysate prepared in RIPA lysis method at 20 mg/ml

Lane 5: Mouse kidney lysate prepared in RIPA lysis method at 20 μg

Lanes 6-7 : Mouse spleen lysate prepared in spleen lysis method at 20 μg

Lane 8: Rat brain lysate prepared in RIPA lysis method at 20 µg

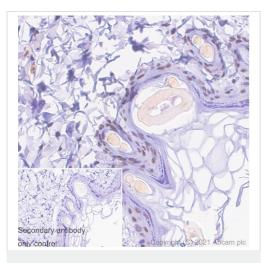
Lane 9: Rat kidney lysate prepared in RIPA lysis method at 20 µg

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 64 kDa **Observed band size:** 64 kDa

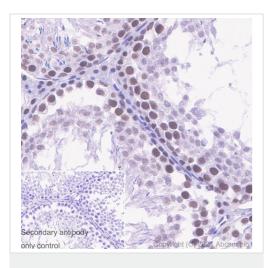


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded Rat skin tissue labeling METTL3 using ab195352 at 1/2000 dilution, followed by a Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstain: Hematoxylin. Nuclear staining on Rat skin. The section was incubated with ab195352 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Perform Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Inset image: negative control - secondary antibody only.

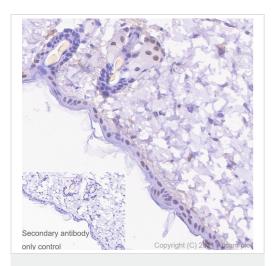


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling METTL3 using ab195352 at 1/2000 dilution, followed by a Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstain: Hematoxylin. Nuclear staining on Rat testis. The section was incubated with ab195352 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Perform Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Inset image: negative control - secondary antibody only.

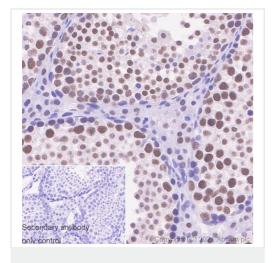


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded Mouse skin labeling METTL3 using ab195352 at 1/2000 dilution, followed by a Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstain: Hematoxylin. Nuclear staining on Mouse skin. The section was incubated with ab195352 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Perform Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Inset image: negative control - secondary antibody only.

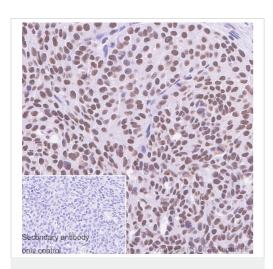


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling METTL3 using ab195352 at 1/2000 dilution, followed by a Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstain: Hematoxylin. Nuclear staining on Mouse testis. The section was incubated with ab195352 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Perform Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Inset image: negative control - secondary antibody only.

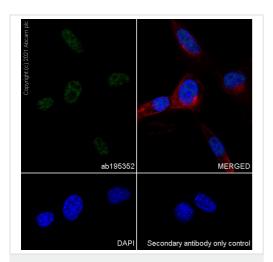


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded Human ovarian cancer tissue labeling METTL3 using ab195352 at 1/2000 dilution, followed by a Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counterstain: Hematoxylin. Nuclear staining on human ovarian cancer. The section was incubated with ab195352 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

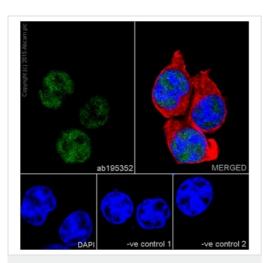
Perform Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Inset image: negative control - secondary antibody only.



Immunocytochemistry/ Immunofluorescence - Anti-METTL3 antibody [EPR18810] (ab195352)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling METTL3 with ab195352 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in NIH/3T3 cells. The nuclear counter stain is DAPI (blue). Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) ab195889 was used at 1/200 dilution as the counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-METTL3 antibody [EPR18810] (ab195352)

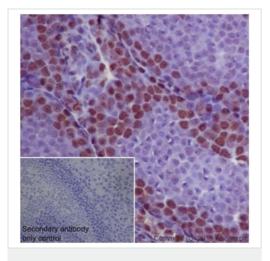
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT116 (Human colorectal carcinoma cell line) cells labeling METTL3 with ab195352 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HCT116 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with anti-alpha Tubulin mouse mAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (AlexaFluor®594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

1. ab195352 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (AlexaFluor®594) (ab150120) secondary antibody at 1/1000 dilution.

2. anti-alpha Tubulin mouse mAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

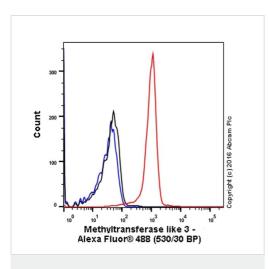


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

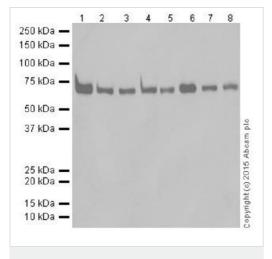
Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling METTL3 using ab195352 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Counterstain: Hematoxylin. Inset image: negative control obtained using PBS instead of ab195352, and secondary antibody only.

Note: Nuclear staining on rat testis was observed.

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



Flow Cytometry (Intracellular) - Anti-METTL3 antibody [EPR18810] (ab195352)



Western blot - Anti-METTL3 antibody [EPR18810] (ab195352)

ab195352 staining METTL3 in the human cell line HEK-293 (Human epithelial cell line from embryonic kidney) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a 1/70 dilution, followed by Goat-Anti Rabbit IgG (Alexa Fluor[®] 488) secondary antibodyat a1/2000 dilution.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

All lanes : Anti-METTL3 antibody [EPR18810] (ab195352) at 1/5000 dilution

Lane 1: Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lane 3: HEK-293 (Human epithelial cells from embryonic kidney)

whole cell lysate

Lane 4: Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lane 5 : NCCIT (human pluripotent embryonal carcinoma) whole cell lysate

Lane 6: F9 (Mouse embyro testicular cancer cell line) whole cell lysate

Lane 7: Neuro-2a (Mouse neuroblastoma cells) whole cell lysate

Lane 8: LLC1 (Mouse lung carcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 64 kDa

Observed band size: 64 kDa

Exposure time: 10 seconds

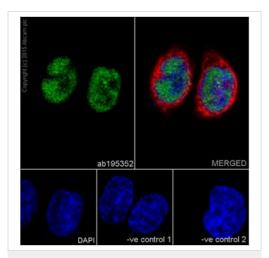
5% NFDM/TBST: Blocking and diluting buffer.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody [EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling METTL3 using ab195352 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Counterstain: Hematoxylin. Inset image: negative control obtained using PBS instead of ab195352, and secondary antibody only.

Note: Nuclear staining on human bladder cancer was observed.

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



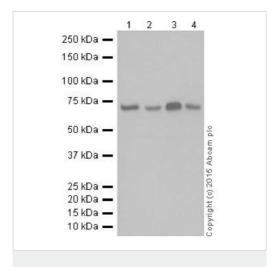
Immunocytochemistry/ Immunofluorescence - Anti-METTL3 antibody [EPR18810] (ab195352)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling METTL3 with ab195352 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

- 1. ab195352 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.
- 2. Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Western blot - Anti-METTL3 antibody [EPR18810] (ab195352)

All lanes : Anti-METTL3 antibody [EPR18810] (ab195352) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysate

Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lane 4: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

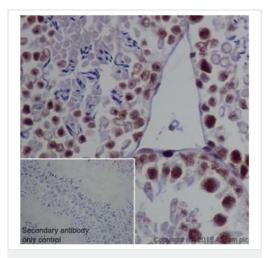
Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/100000 dilution

Predicted band size: 64 kDa **Observed band size:** 64 kDa

Exposure time: 2 seconds

5% NFDM/TBST: Blocking and diluting buffer.



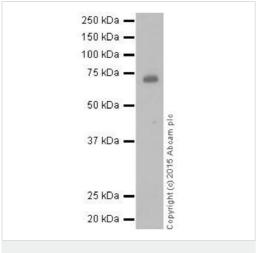
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-METTL3 antibody
[EPR18810] (ab195352)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling METTL3 using ab195352 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Counterstain: Hematoxylin.

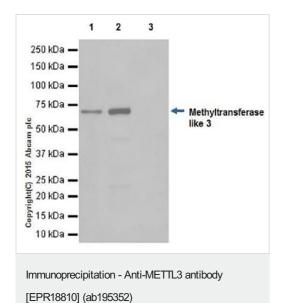
Inset image: negative control obtained using PBS instead of ab195352, and secondary antibody only.

Note: Nuclear staining on mouse testis was observed.

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



Western blot - Anti-METTL3 antibody [EPR18810] (ab195352)



Anti-METTL3 antibody [EPR18810] (ab195352) at 1/5000 dilution + Human thymus lysate at 10 µg

Secondary

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/10000 dilution

Predicted band size: 64 kDa **Observed band size:** 64 kDa

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.

METTL3 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab195352 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab195352 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

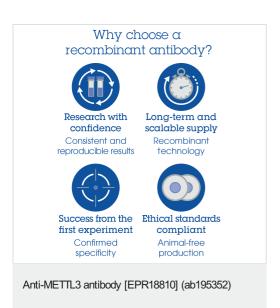
Lane 1: Input: 10µg of HeLa whole cell lysate.

Lane 2: HeLa whole cell lysate following IP with ab195352.

Lane 3: Negative control: IP using Rabbit monoclonal IgG (ab172730) instead of ab195352 in HeLa whole cell lysates.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. 1

second exposure.



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