abcam

Product datasheet

Anti-KMT6 / EZH2 antibody [EPR25353-284] ab307646

敲除验证 重组 RabMAb

19 图像

概述

产品名称	Anti-KMT6 / EZH2抗体[EPR25353-284]
描述	兔单克隆抗体[EPR25353-284] to KMT6 / EZH2
宿主	Rabbit
经测试应用	**适用于:** WB, IHC-P, ICC/IF, IP, ChIP, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Wild-type HAP1, B16-F10, Neuro-2a, HeLa, 293T, NIH/3T3, PC-12 and NCCIT lysates. IHC-P: Human colon, Human pancreatic adenocarcinoma, Mouse colon, Mouse pancreatic tumor, Rat colon and Wild-type HAP1 tissues. ICC/IF: Wild-type HAP1, NCCIT and B16-F10 cells. Flow Cyt (Intra): Wild-type HAP1, NCCIT and B16-F10 cells. IP: NCCIT and B16-F10 cells. ChIP: NCCIT cells
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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR25353-284
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab307646于以下的经测试应用**

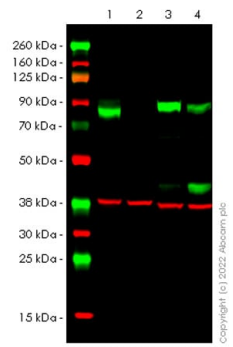
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/1000. Detects a band of approximately 85 kDa (predicted molecular weight: 85 kDa).
IHC-P		1/100 - 1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/50.
IP		1/30.
ChIP		Use a concentration of 5 µg/ml.
Flow Cyt (Intra)		1/500.

靶标

功能	Polycomb group (PcG) protein. Catalytic subunit of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' and 'Lys-27' of histone H3, leading to transcriptional repression of the affected target gene. Able to mono-, di- and trimethylate 'Lys-27' of histone H3 to form H3K27me1, H3K27me2 and H3K27me3, respectively. Compared to EZH2-containing complexes, it is more abundant in embryonic stem cells and plays a major role in forming H3K27me3, which is required for embryonic stem cell identity and proper differentiation. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes.
组织特异性	Expressed in many tissues. Overexpressed in numerous tumor types including carcinomas of the breast, colon, larynx, lymphoma and testis.
序列相似性	Belongs to the histone-lysine methyltransferase family. EZ subfamily. Contains 1 SET domain.
发展阶段	Expression decreases during senescence of embryonic fibroblasts (HEFs). Expression peaks at the G1/S phase boundary.
翻译后修饰	Phosphorylated by AKT1. Phosphorylation by AKT1 reduces methyltransferase activity.
细胞定位	Nucleus.

图片



Western blot - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

All lanes : Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646) at 1/1000 dilution

Lane 1 : Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell line) whole cell lysate
Lane 2 : KMT6 / EZH2 knockout HAP1 whole cell lysate
Lane 3 : B16-F10 (mouse skin melanoma cell) whole cell lysate
Lane 4 : Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

Predicted band size: 85 kDa
Observed band size: 85 kDa

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

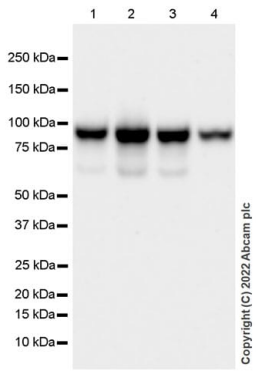
The samples were run on a Bis-Tris gel.

Performed under reducing conditions.

False colour image of Western blot: Anti-KMT6 / EZH2 antibody (ab307646) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab307646 was shown to bind specifically to KMT6 / EZH2. A band was observed at 85kDa in wild-type HAP1 cell lysates whereas no signal observed at this size in KMT6 / EZH2 knockout cell line. To generate this image, wild-type and KMT6 / EZH2 knockout HAP1 cell lysates were analyzed. First, samples were run on a Bis-Tris gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in in Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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 IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse

IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

All lanes : Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervical adenocarcinoma) whole cell lysate
Lane 2 : 293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 4 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

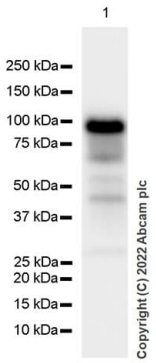
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 85 kDa
Observed band size: 85 kDa

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

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

Exposure time: 26 seconds



Western blot - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646) at 1/1000 dilution + NCCIT (human pluripotent embryonic carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

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

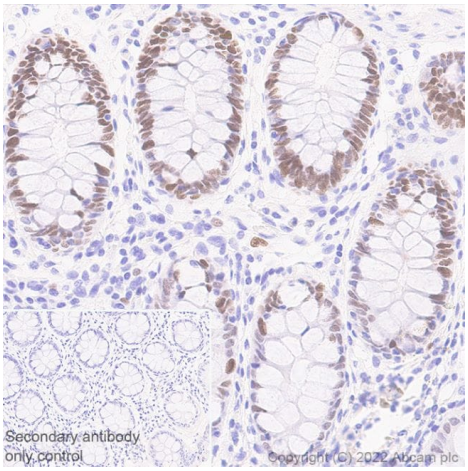
Predicted band size: 85 kDa

Observed band size: 85 kDa

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

The bands beneath the target band (85 kDa) are likely to be degraded target fragments.

Exposure time: 3 seconds

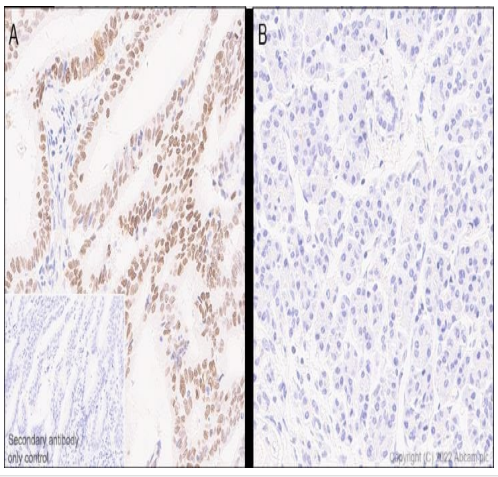


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling KMT6 / EZH2 with ab307646 at 1/100 (5.43 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human colon. The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

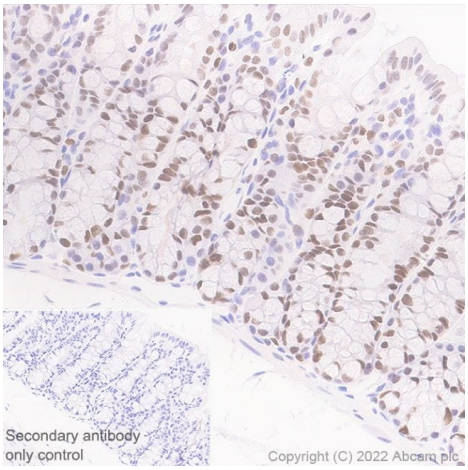


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded Human pancreatic adenocarcinoma and adjacent tissue labeling KMT6 / EZH2 with ab307646 at 1/100 (5.43 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human pancreatic adenocarcinoma (image A) and no staining on the adjacent tissue (image B). The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

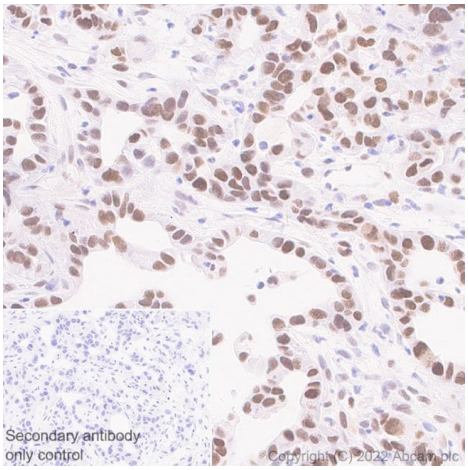


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling KMT6 / EZH2 with ab307646 at 1/1000 (0.543 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on mouse colon. The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

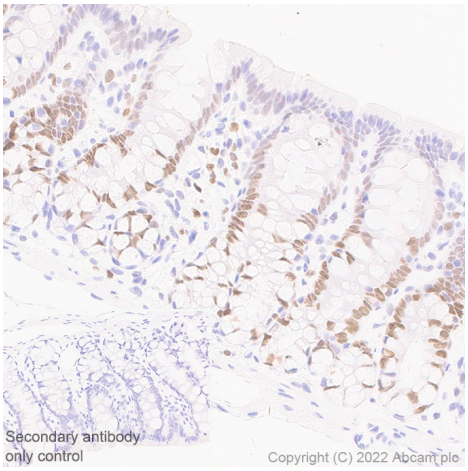


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded Mouse pancreatic tumor tissue labeling KMT6 / EZH2 with ab307646 at 1/1000 (0.543 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on mouse pancreatic tumor. The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

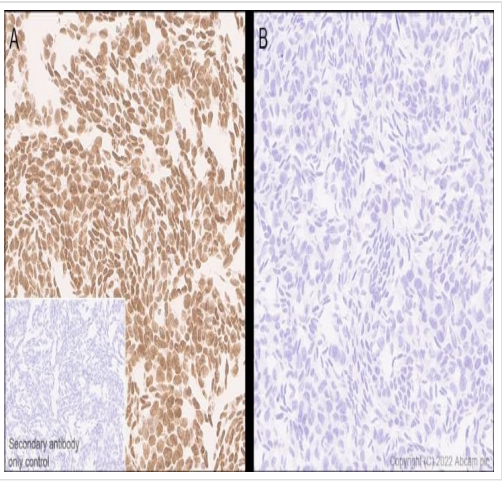


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling KMT6 / EZH2 with ab307646 at 1/1000 (0.543 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on rat colon. The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

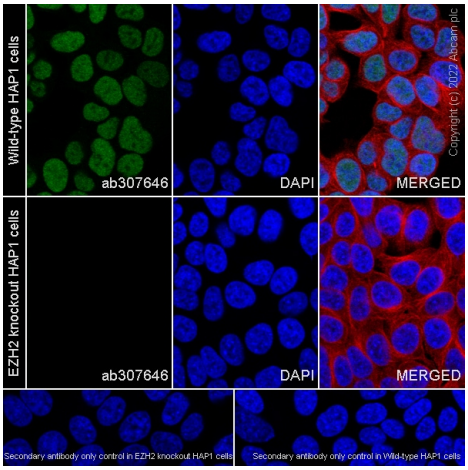


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunohistochemical analysis of paraffin-embedded A) Wild-type HAP1 (Human chronic myelogenous leukemia near-haploid cell) cell pellet and B) EZH2 knockout HAP1 cell pellet labeling KMT6 / EZH2 with ab307646 at 1/1000 (0.543 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on (A) wild-type HAP1 cell pellet, no staining on (B) EZH2 knockout HAP1 cell pellet. The section was incubated with ab307646 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

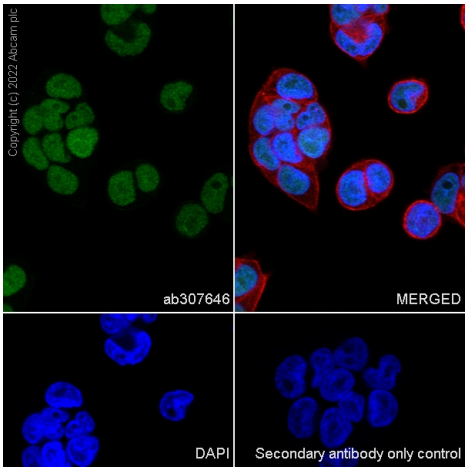
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized EZH2 KO HAP1 (EZH2 knockout human chronic myelogenous leukemia) cells labelling KMT6 / EZH2 with ab307646 at 1/50 (10.86 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2ug/ml) dilution (Green). Confocal image showing nuclear staining in Parental HAP1 cell line, and no taining in EZH2 KO HAP1 cell line. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

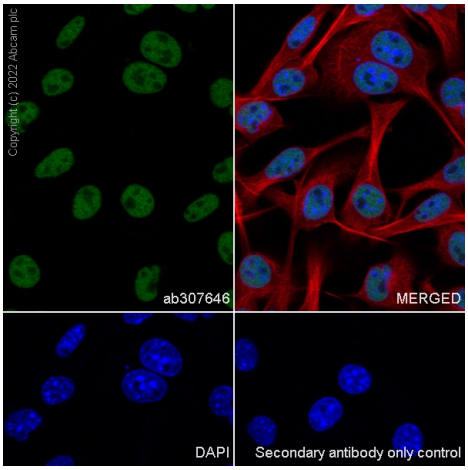
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/ml) dilution.



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NCCIT (human pluripotent embryonic carcinoma epithelial cell) cells labelling KMT6 / EZH2 with ab307646 at 1/50 (10.86 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2ug/ml) dilution (Green). Confocal image showing nuclear staining in NCCIT cell line .Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

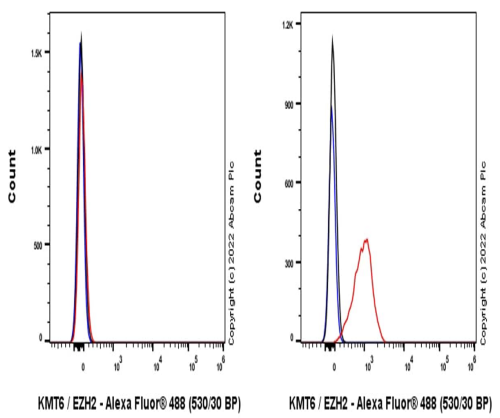
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/ml) dilution.



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

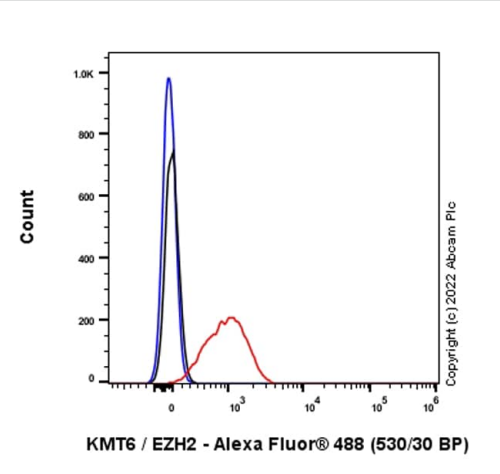
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized B16-F10 (mouse skin melanoma cell) cells labelling KMT6 / EZH2 with ab307646 at 1/50 (10.86 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2ug/ml) dilution (Green). Confocal image showing nuclear staining in B16-F10 cell line. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/ml) dilution.



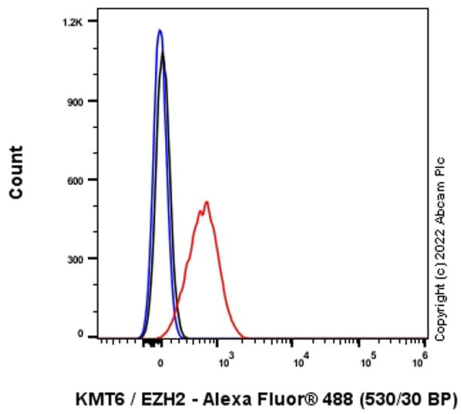
Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell, Right) / KMT6 knockout HAP1(Left) cells labelling KMT6 / EZH2 with ab307646 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, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



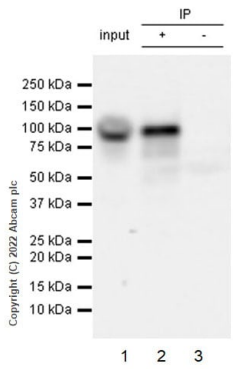
Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NCCIT (human pluripotent embryonic carcinoma epithelial cell) cells labelling KMT6 / EZH2 with ab307646 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, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized B16-F10 (Mouse skin melanoma) cells labelling KMT6 / EZH2 with ab307646 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, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

KMT6 / EZH2 was immunoprecipitated from 0.35 mg NCCIT (human pluripotent embryonic carcinoma epithelial cell) whole cell lysate 10 ug with ab307646 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab307646 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: NCCIT (human pluripotent embryonic carcinoma epithelial cell) whole cell lysate 10 ug

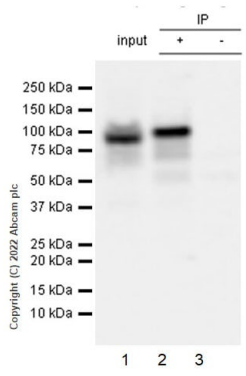
Lane 2: ab307646 IP in NCCIT whole cell lysate

Lane 3:Rabbit monoclonal IgG (**ab172730**) instead of ab307646 in NCCIT whole cell lysate

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

Exposure time: 5 seconds

Lysate was freshly made and used for IP immediately to minimize protein degradation.



Immunoprecipitation - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

KMT6 / EZH2 was immunoprecipitated from 0.35 mg B16-F10 (mouse skin melanoma cell) whole cell lysate 10 ug with ab307646 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab307646 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: B16-F10 (mouse skin melanoma cell) whole cell lysate 10 ug

Lane 2: ab307646 IP in B16-F10 whole cell lysate

Lane 3:Rabbit monoclonal IgG (**ab172730**) instead of ab307646 in B16-F10 whole cell lysate

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

Exposure time: 5 seconds

Lysate was freshly made and used for IP immediately to minimize protein degradation.

ChIP - Anti-KMT6 / EZH2 antibody [EPR25353-284] (ab307646)

Chromatin was prepared from NCCIT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab307646(red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Anti-KMT6 / EZH2 antibody [EPR25353-284]

(ab307646)

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