abcam

Product datasheet

Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal ab191080

敲除验证 重组 RabMAb

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概述

产品名称	Anti-KMT6 / EZH2抗体[EPR9307(2)] - N-terminal
描述	兔单克隆抗体[EPR9307(2)] to KMT6 / EZH2 - N-terminal
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IHC-P **不适用于:** ChIP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	Human breast carcinoma tissue; HeLa cells. WB: HEK293 cell lysate, Ms testis tissue lysate, mouse and rat spleen tissue 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: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR9307(2)

同种型 IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab191080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
Flow Cyt (Intra)		1/90. Purified format. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/500. Detects a band of approximately 93 kDa (predicted molecular weight: 85 kDa).
ICC/IF	★★☆☆☆ (1)	1/250.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

应用说明 Is unsuitable for ChIP.

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.

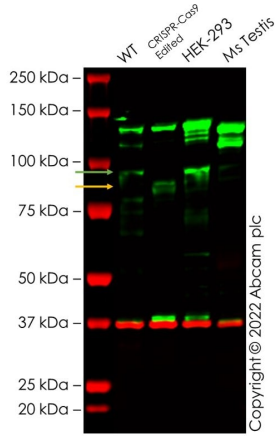
序列相似性 Belongs to the histone-lysine methyltransferase family. EZ subfamily.
Contains 1 SET domain.

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 [EPR9307(2)] - N-terminal (ab191080)

All lanes : Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080) at 1/500 dilution

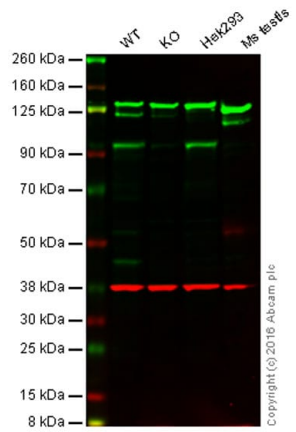
Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : ezh2 CRISPR-Cas9 edited MCF7 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : Mouse Testis cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 90 kDa

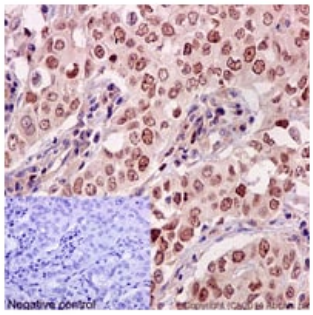
False colour image of Western blot: Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab191080 was shown to bind specifically to KMT6 / EZH2. A band was observed at 90 kDa (green arrow) in wild-type MCF7 cell lysates with no signal observed at this size in ezh2 CRISPR-Cas9 edited cell line **ab281611** (CRISPR-Cas9 edited cell lysate **ab282963**). The band observed in the CRISPR-Cas9 edited lysate lane below 90 kDa (yellow arrow) is likely to represent a truncated form of KMT6 / EZH2. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and ezh2 CRISPR-Cas9 edited MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: KMT6 / EZH2 knockout HAP1 cell lysate (20 µg)
Lane 3: HEK293 cell lysate (20 µg)
Lane 4: Ms testis tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab191080 observed at 93 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

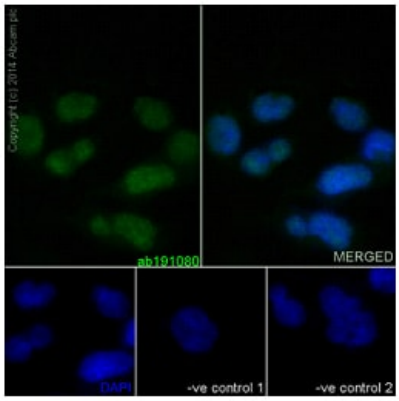
ab191080 was shown to recognize KMT6 / EZH2 when KMT6 / EZH2 knockout samples were used, along with additional cross-reactive bands. Wild-type and KMT6 / EZH2 knockout samples were subjected to SDS-PAGE. ab191080 and **ab8245** (loading control to GAPDH) were diluted at 1/500 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

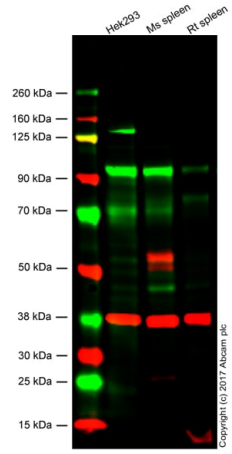
Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling KMT6 / EZH2 with ab191080 at 1/250 dilution followed by pre-diluted HRP Polymer for Rabbit IgG secondary antibody and counter-stained with Hematoxylin (inset: negative control).

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



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

Immunofluorescent analysis of HeLa cells (4% Paraformaldehyde-fixed; 0.1% tritonX-100-permeabilized) labeling KMT6 / EZH2 with ab191080 at 1/250 dilution followed by Goat anti rabbit IgG (AlexaFluor® 488) secondary at 1/200 dilution and counter-stained with DAPI (blue).



Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

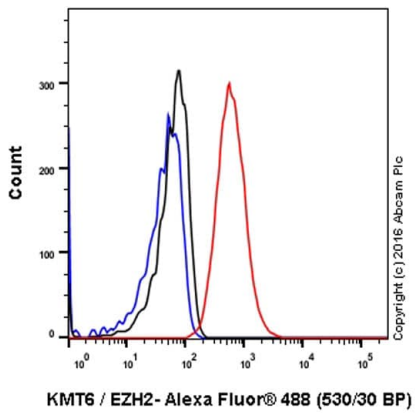
Lane 1: HEK293 cell lysate (20 µg)

Lane 2: Mouse spleen tissue lysate (20 µg)

Lane 3: Rat spleen tissue lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab191080 observed at 93 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Human, mouse and rat extracts were subjected to SDS-PAGE. ab191080 and **ab8245** (loading control to GAPDH) were diluted at 1/500 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

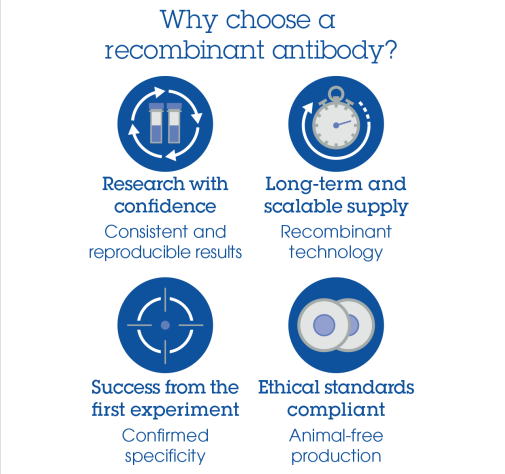


Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

ab191080 staining KMT6 / EZH2in the human cell line Jurkat (human acute T cell leukemia) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/90. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

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



Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

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