abcam

Product datasheet

Anti-Bmi1 antibody [EPR3745(2)] ab126783

敲除 验证　重组　RabMAb®

★★★☆☆ **3 Abreviews**　**37 References**　16 图像

概述

产品名称	Anti-Bmi1抗体[EPR3745(2)]
描述	兔单克隆抗体[EPR3745(2)] to Bmi1
宿主	Rabbit
经测试应用	**适用于:** IP, ChIC/CUT&RUN-seq, WB, IHC-P, ICC/IF
种属反应性	**与反应:** Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: MCF7, A431, HEK293T, K562, SAOS-2, SW480, MOLT4, PC-12 and HT1080 cell lysates. IHC-P: Human tonsil, colonic adenocarcinoma, lung adenocarcinoma, breast carcinoma and thyroid gland carcinoma tissues. ICC/IF: SW480 and HeLa cells. IP: K-562 cell lysate ChIC/CUT&RUN-Seq: NCCIT cells.
常规说明	Mouse: Internal data indicated that the antibody is not suitable for WB application in mouse species. 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. Stable for 12 months at -20°C.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified

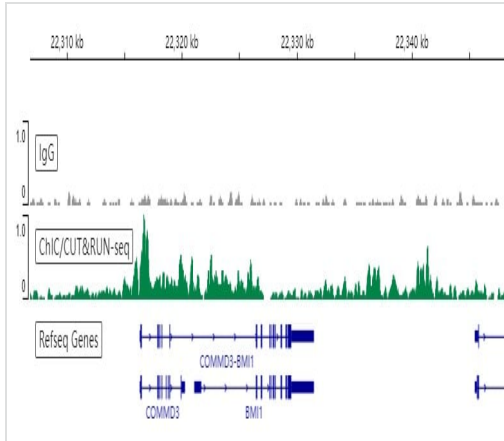|	
克隆	单克隆
克隆编号	EPR3745(2)
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab126783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
IP		1/50.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 μg
WB	★★★☆☆ **(2)**	1/10000 - 1/50000. Detects a band of approximately 40 kDa (predicted molecular weight: 36 kDa).
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		1/100 - 1/500.

靶标

功能	Component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility. In the PRC1 complex, it is required to stimulate the E3 ubiquitin-protein ligase activity of RNF2/RING2.
序列相似性	Contains 1 RING-type zinc finger.
翻译后修饰	Monoubiquitinated (By similarity). May be polyubiquitinated; which does not lead to proteasomal degradation.
细胞定位	Nucleus. Cytoplasm.

图片

ChIC/CUT&RUN sequencing - Anti-Bmi1 antibody
[EPR3745(2)] (ab126783)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 NCCIT (Human pluripotent embryonic carcinoma cell line) cells and 5 µg of ab126783 [EPR3745(2)]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

Western blot - Anti-Bmi1 antibody [EPR3745(2)]
(ab126783)
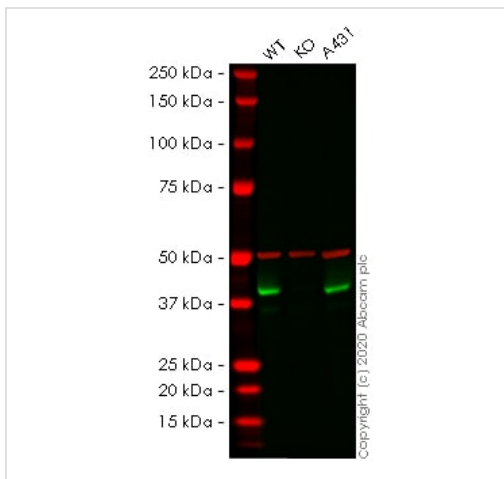
All lanes : Anti-Bmi1 antibody [EPR3745(2)] (ab126783) at 1/10000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : BMI1 knockout MCF7 cell lysate
Lane 3 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 36 kDa
Observed band size: 37 kDa

Lanes 1- 3: Merged signal (red and green). Green - ab126783 observed at 37 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab126783 was shown to react with Bmi1 in wild-type MCF7 cells in western blot. Loss of signal was observed when knockout cell line **ab262319** (knockout cell lysate **ab256851**) was used. Wild-type MCF7 and BMI1 knockout MCF7 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room

temperature in 0.1% TBST with 3% non-fat dried milk. ab126783 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.
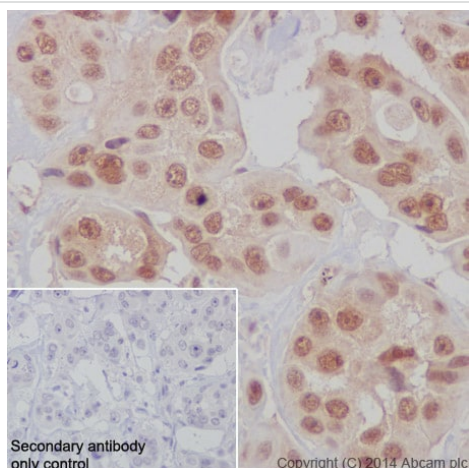
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling Bmi1 with purified ab126783 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
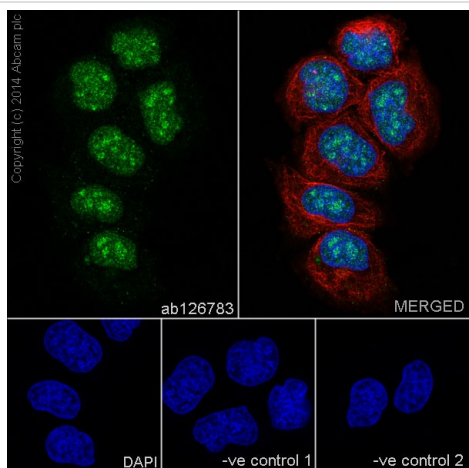

Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Bmi1 with purified ab126783 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/500) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).
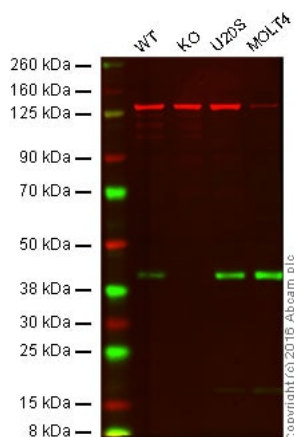
Western blot - Anti-Bmi1 antibody [EPR3745(2)]
(ab126783)
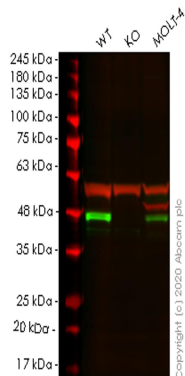
Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: Bmi1 knockout HAP1 cell lysate (20 µg)

Lane 3: U2OS cell lysate (20 µg)

Lane 4: Molt-4 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab126783 observed at 42 kDa. Red - loading control, **ab18058**, observed at 37 kDa.

ab126783 was shown to specifically react with Bmi1 when Bmi1 knockout samples were used. Wild-type and Bmi1 knockout samples were subjected to SDS-PAGE. ab126783 and **ab18058** (loading control to Vinculin) were both diluted at 1/10 000 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.

Western blot - Anti-Bmi1 antibody [EPR3745(2)]
(ab126783)

All lanes : Anti-Bmi1 antibody [EPR3745(2)] (ab126783) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : BMI1 knockout HEK293T cell lysate

Lane 3 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 36 kDa

Observed band size: 37 kDa

Lanes 1-3: Merged signal (red and green). Green - ab126783 observed at 37 kDa. Red - loading control **ab7291** observed at 50 kDa.
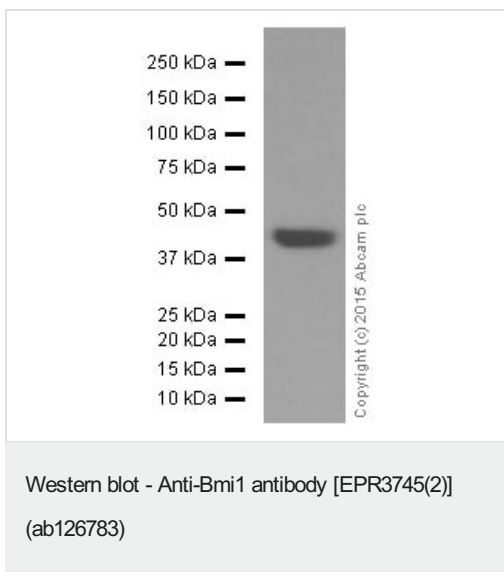
 ab126783 Anti-Bmi1 antibody [EPR3745(2)] was shown to

specifically react with Bmi1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266514** (knockout cell lysate **ab256850**) was used. Wild-type and Bmi1 knockout samples were subjected to SDS-PAGE. ab126783 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

Immunoprecipitation - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)
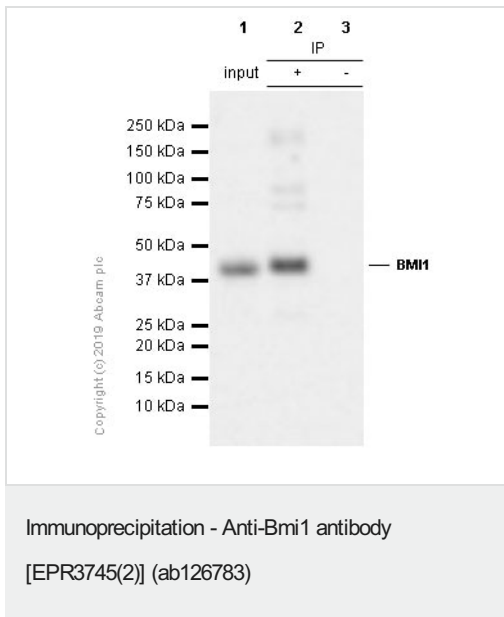
ab126783 (purified) at 1/50 immunoprecipitating Bmi1 in 10 µg K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate (**Lanes 1 and 2**, observed at 43 kDa). **Lane 3** - Rabbit monoclonal IgG (**ab172730**) instead of ab126783 in K-562 whole cell lysate. For western blotting, ab126783 at 1/500 and VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

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

Western blot - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

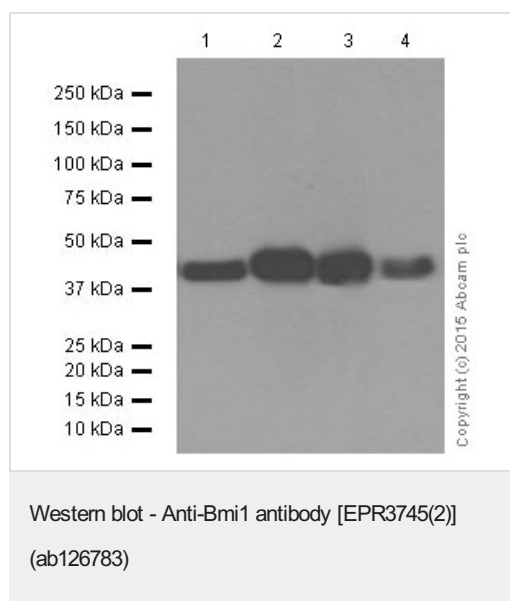Anti-Bmi1 antibody [EPR3745(2)] (ab126783) at 1/20000 dilution (purified) + PC-12 cell lysate at 20 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 36 kDa
Observed band size: 40 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Western blot - Anti-Bmi1 antibody [EPR3745(2)]
(ab126783)

All lanes : Anti-Bmi1 antibody [EPR3745(2)] (ab126783) at
1/20000 dilution (purified)

Lane 1 : K562 cell lysate

Lane 2 : SAOS-2 cell lysate

Lane 3 : SW480 cell lysate

Lane 4 : Molt-4 cell lysate
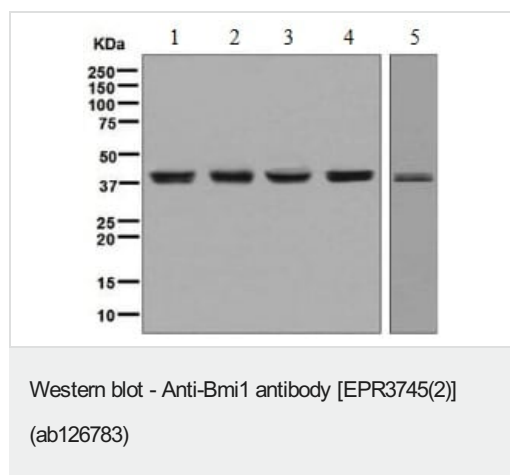
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at
1/1000 dilution

Predicted band size: 36 kDa

Observed band size: 40 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Western blot - Anti-Bmi1 antibody [EPR3745(2)]
(ab126783)

All lanes : Anti-Bmi1 antibody [EPR3745(2)] (ab126783) at
1/10000 dilution (unpurified)

Lane 1 : K562 cell lysate

Lane 2 : SAOS-2 cell lysate

Lane 3 : SW480 cell lysate

Lane 4 : MOLT4 cell lysate

Lane 5 : HT1080 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

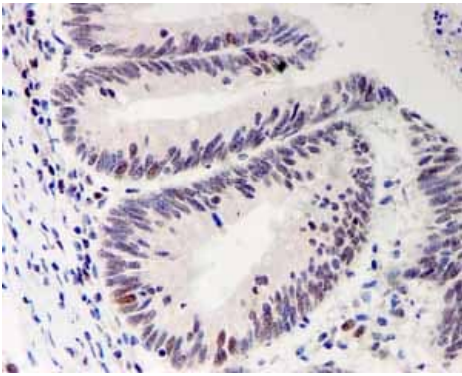**All lanes :** HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 36 kDa
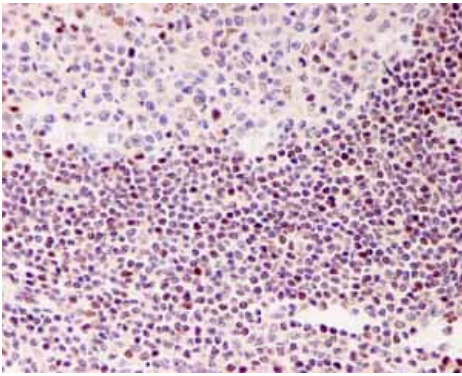
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human normal tonsil tissue labelling Bmi1 with unpurifiied ab126783.
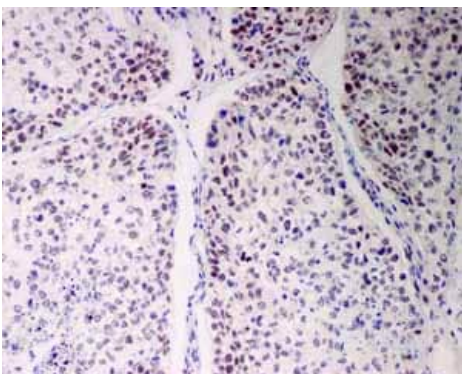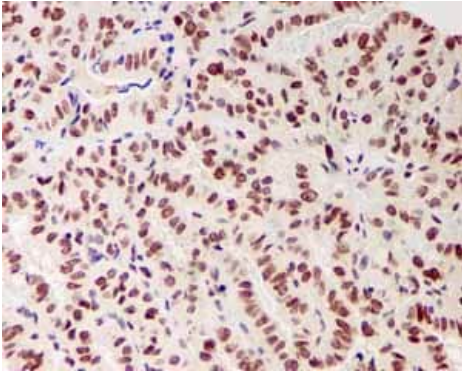
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic adenocarcinoma tissue labelling Bmi1 with unpurifiied ab126783.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung adenocarcinoma tissue labelling Bmi1 with unpurifiied ab126783.
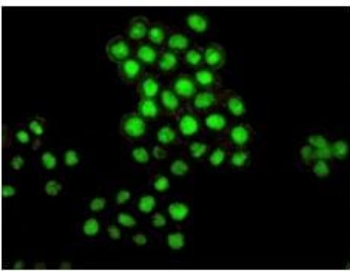
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid gland carcinoma tissue labelling Bmi1 with unpurifiied ab126783.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Immunocytochemistry/Immunofluorescence analysis of SW480 cells labelling Bmi1 with unpurified ab126783 at a dilution of 1/100.

Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

Anti-Bmi1 antibody [EPR3745(2)] (ab126783)

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