abcam

Product datasheet

Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free ab216444

KO VALIDATED Recombinant RabMAb

5 References 13 Images

Overview

Product name	Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR3745(2)] to Bmi1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, IHC-P, WB, ICC/IF, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab216444 is the carrier-free version of **ab126783**. Mouse: Internal data indicated that the antibody is not suitable for WB application in mouse species. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3745(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab216444 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

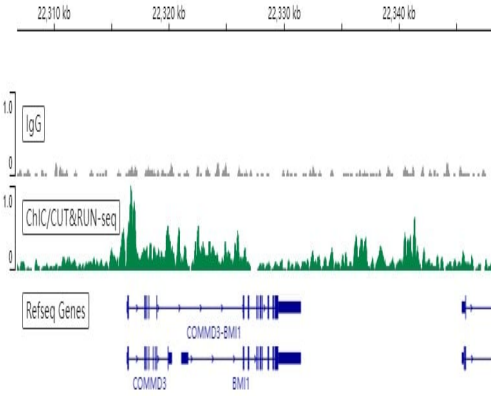
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Detects a band of approximately 40 kDa (predicted molecular weight: 36 kDa).
ICC/IF		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.

Target

Function 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.

Sequence similarities	Contains 1 RING-type zinc finger.
Post-translational modifications	Monoubiquitinated (By similarity). May be polyubiquitinated; which does not lead to proteasomal degradation.
Cellular localization	Nucleus. Cytoplasm.

Images



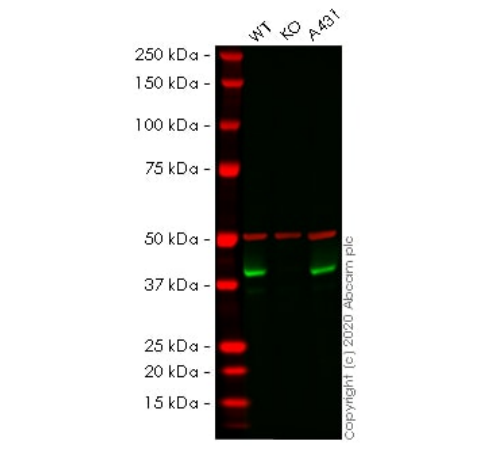
ChIC/CUT&RUN sequencing - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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.

This data was developed using the same antibody clone in a different buffer formulation (**ab126783**).



Western blot - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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.

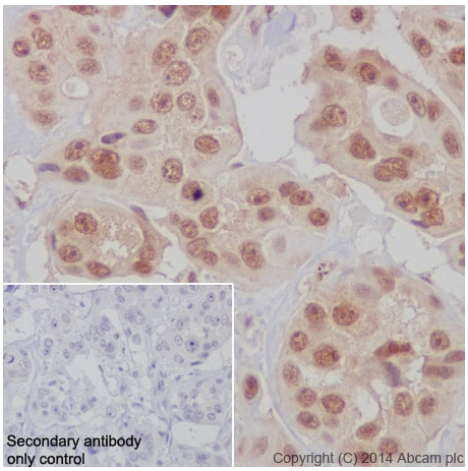
Predicted band size: 36 kDa
Observed band size: 37 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab126783**).

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

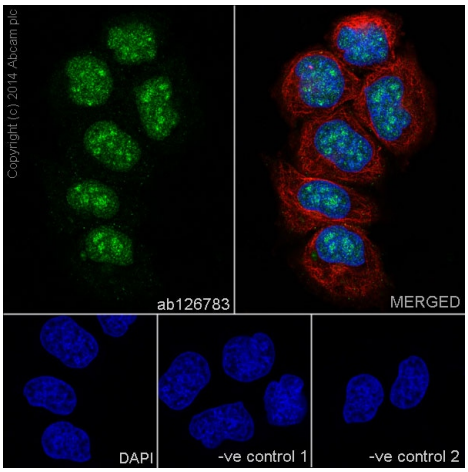
western blot. Loss of signal was observed when knockout cell line **ab262319** (knockout cell lysate **ab256851**) was used. Wild-type MCF7 and BMI1 knockout MCF7 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab126783** 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)] - BSA and Azide free (ab216444)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).



Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

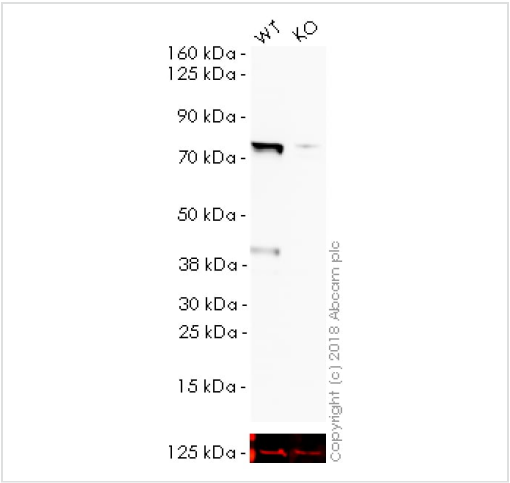
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Bmi1 with purified **ab126783** at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody.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

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).



Western blot - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

All lanes : Anti-Bmi1 antibody [EPR3745(2)] (HRP) (**ab197620**) at 1/1000 dilution

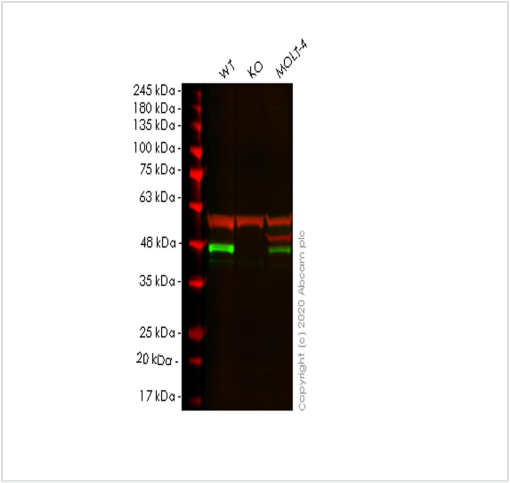
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : COMMD3-BMI1 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 36 kDa

ab197620 was shown to recognize Bmi1 in wild-type HAP1 cells as signal was lost at the expected MW in COMMD3-BMI1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and COMMD3-BMI1 knockout samples were subjected to SDS-PAGE. Ab197620 and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197620**).



Western blot - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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.

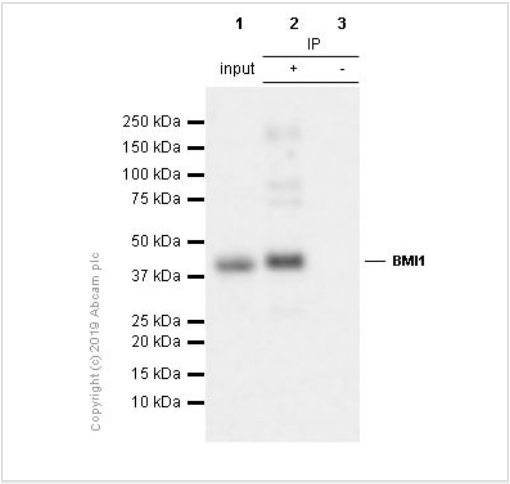
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

This data was developed using the same antibody clone in a different buffer formulation (**ab126783**).

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.

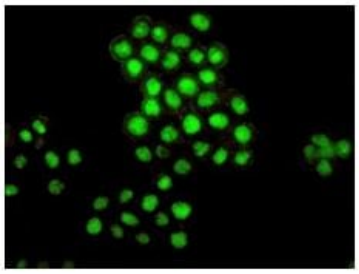


Immunoprecipitation - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

ab126783 (purified) at 1/500 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, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

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

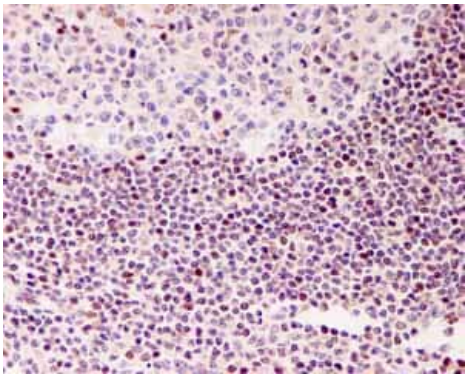
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).



Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).

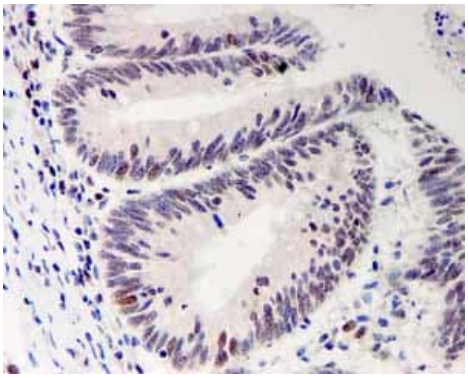


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).

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

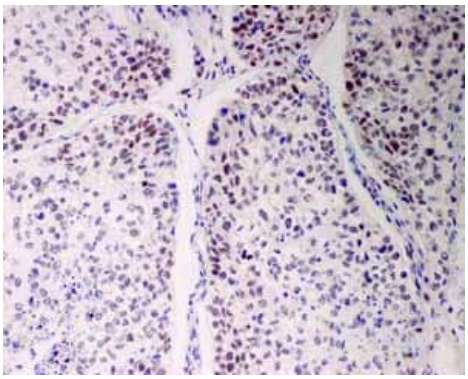


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).

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

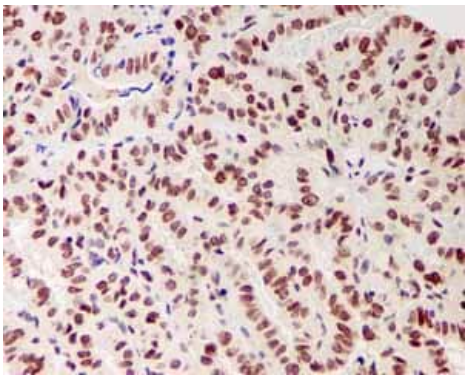


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).

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

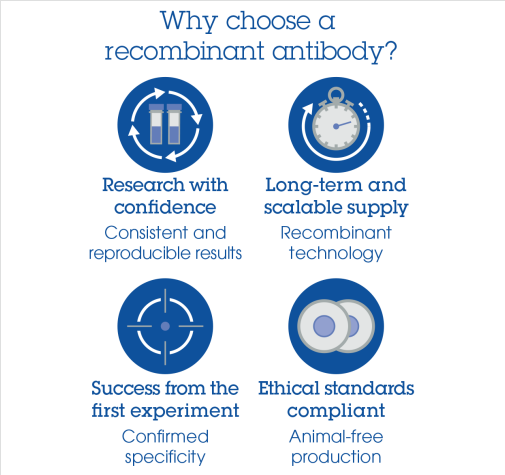


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126783**).

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



Anti-Bmi1 antibody [EPR3745(2)] - BSA and Azide free (ab216444)

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