abcam

ab254253

Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR22604-160
Purification technique	Affinity purification Protein A
Concentration	0.5845 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/5000
Notes	-

Species	Mouse

Dilution info	1/5000
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	5 µg
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	for 10^7 Cells

Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	5 µg

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Species	Human
Dilution info	1/50
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/60
Notes	-

Species	Human
Dilution info	1/60
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

Target data

See full target information BMI1

Function Component of a Polycomb group (PcG) multiprotein PRC1-like complex, a complex class 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 (PubMed:15386022, PubMed:16359901, PubMed:16714294, PubMed:21772249, PubMed:25355358, PubMed:26151332, PubMed:27827373). The complex composed of RNF2, UB2D3 and BMI1 binds nucleosomes, and has activity only with nucleosomal histone H2A (PubMed:21772249, PubMed:25355358). In the PRC1-like complex, regulates the E3 ubiquitin-protein ligase activity of RNF2/RING2 (PubMed:15386022, PubMed:21772249, PubMed:26151332).

Storage

Shipped at conditions Conditional Ambient

Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Bmi1 also known as B-cell-specific Moloney murine leukemia virus integration site 1 is a member of the Polycomb group of proteins. It has an approximate molecular mass of 37 kDa. Bmi1 is expressed in many tissues including the brain bone marrow and hematopoietic cells. It functions as a transcriptional repressor. Bmi1 plays an important role in chromatin remodeling influencing gene silencing and regulation of developmental processes. Bmi1 ELISA assays are commonly used for quantifying its levels in various research applications.
Biological function summary	The functions of Bmi1 extend into maintaining stem cell renewal and differentiation. It acts as part of the Polycomb repressive complex 1 (PRC1) partnering with other proteins to regulate gene expression by altering chromatin structure. Bmi1 protein regulates cell proliferation and inhibits senescence by repressing the INK4a/ARF locus which encodes tumor suppressor proteins like p16INK4a and p14ARF.
Pathways	Bmi1 plays significant roles in processes such as the DNA damage response and the self-renewal of hematopoietic stem cells. It is involved in the modulation of the

	Wnt and Notch signaling pathways. Bmi1 protein interacts closely with proteins such as CBX2 and RNF2 within these pathways affecting the transcriptional landscape that governs cell fate decisions.
Associated diseases and disorders	Bmi1 overexpression links to cancer particularly breast cancer and leukemia. In MCF7 breast cancer cells alterations in Bmi1 activity contribute to oncogenesis and tumor progression. Its dysregulation can connect to Myc a well-known oncogenic protein. Additionally Bmi1's influence in Cas9-based gene editing models highlights its potential in cancer therapy research. Bmi1 has also been implicated in neurodegenerative disorders with connections to proteins like p21CIP1 affecting neuronal survival and aging.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

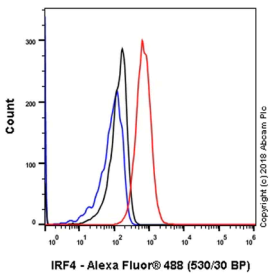
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

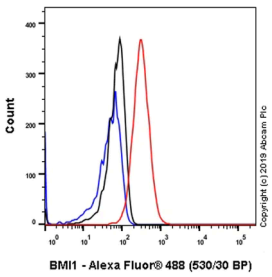
17 product images



Flow Cytometry (Intracellular) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Flow Cytometry (Intracellular) staining using rabbit Anti-Bmi1 antibody

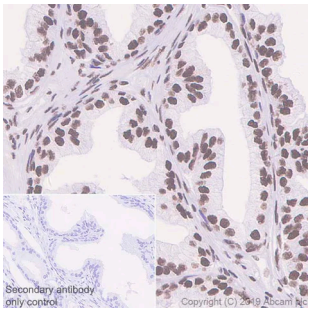
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling Bmi1 with ab254253 at 1/60 (red) compared with a Rabbit monoclonal IgG (ab172730) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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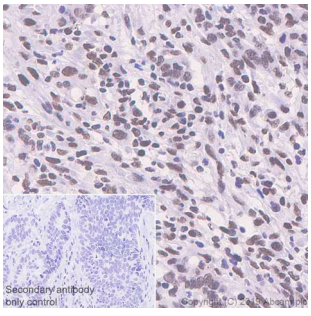
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bmi1 antibody

Immunohistochemical analysis of paraffin-embedded human prostatic hyperplasia tissue labeling Bmi1 with ab254253 at 1/500 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human prostatic hyperplasia is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is the ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab254253 for 30 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

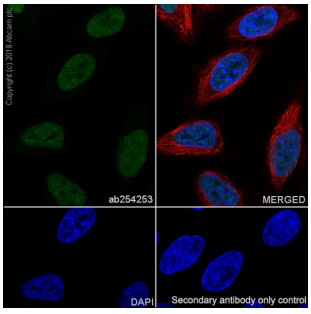
Bmi1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bmi1 antibody

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling Bmi1 with ab254253 at 1/500 dilution, followed by a ready to use Rabbit specific IHC polymer detection

kit HRP/DAB (ab209101). Nuclear staining on human gastric cancer is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is the ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

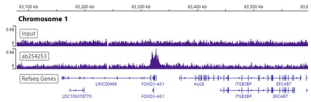
The section was incubated with ab254253 for 30 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

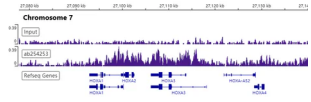
Bmi1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bmi1 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized, HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Bmi1 with ab254253 at 1/50 dilution, followed by a AlexaFluor®488 Goat anti-Rabbit (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in Hela cells. DAPI was used as a nuclear counterstain (blue). Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, ab195889) at 1/200 was used as a counterstain.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a AlexaFluor®488 Goat anti-Rabbit (ab150077) secondary antibody at 1/1000 dilution.



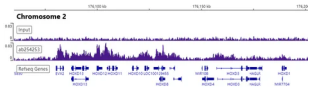
ChIP-sequencing - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Chromatin was prepared from NCCIT cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 NCCIT cells and 8 µg of ab254253 [EPR22604-160]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.
Additional screenshots of mapped reads can be downloaded here.



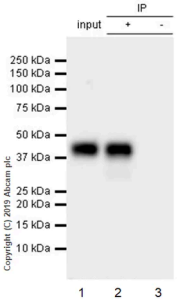
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Additional screenshots of mapped reads can be downloaded here.



Immunoprecipitation - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 was immunoprecipitated from 0.35mg of K562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate with ab254253 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab254253 at 1/1000 dilution. VeriBlot for IP detection reagent (HRP) (ab131366) was used as secondary antibody at 1/5000 dilution.
Lane 1: K562 whole cell lysate 10 µg (Input).

Lane 2: ab254253 IP in K562 whole cell lysate.

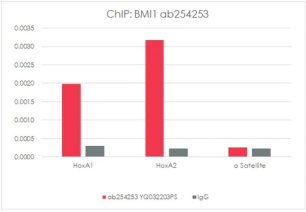
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254253 in K562 whole cell lysate.

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

Exposure time: 15 seconds.

All lanes:
Immunoprecipitation - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

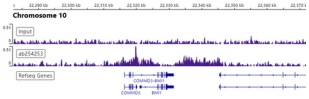
Predicted band size: 36 kDa



ChIP - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

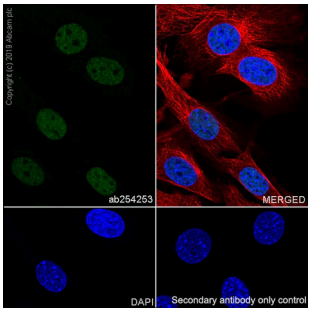
Chromatin was prepared from NCCIT cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab254253 (red), and 20 µl of Protein A/G Sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (sybr green approach).

Primers and probes are located in the first kb of the transcribed region.



ChIP-sequencing - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Chromatin was prepared from NCCIT cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 NCCIT cells and 8 µg of ab254253 [EPR22604-160]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.
Additional screenshots of mapped reads can be downloaded here.

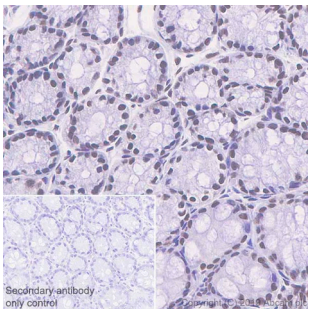


Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bmi1 antibody

Immunofluorescent analysis of 4% paraformaldehyde, 0.1% triton X-100 fixed, NIH/3T3 (mouse embryonic fibroblast) cells labeling Bmi1 with ab254253 at 1/50 dilution, followed by AlexaFluor®488 Goat anti-Rabbit (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in NIH/3T3 cells. DAPI was used as a nuclear counterstain (blue). Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, ab195889) at 1/200 was used as a counterstain.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a AlexaFluor®488 Goat anti-Rabbit (ab150077) secondary antibody at 1/1000 dilution.



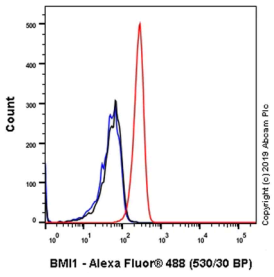
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bmi1 antibody

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Bmi1 with ab254253 at 1/500 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse colon is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is the ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

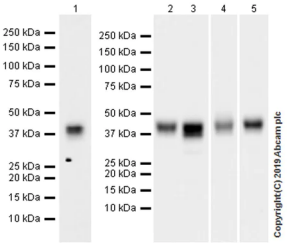
The section was incubated with ab254253 for 30 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Flow Cytometry (Intracellular) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Flow Cytometry (Intracellular) staining using rabbit Anti-Bmi1 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cell line labeling Bmi1 with ab254253 at 1/60 dilution (red) compared with a Rabbit monoclonal IgG (ab172730) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Exposure time:
Lane 1: 48 seconds
Lanes 2-5: 10 seconds
Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Molecular weight observed is consistent with what has been described in the literature (PMID: 26110620)

All lanes:
Western blot - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253) at 1/5000 dilution

Lane 1:
K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate at 20 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
MOLT-4 (human lymphoblastic leukemia T lymphoblast), whole cell lysate at 20 µg

Lane 4:
A549 (human lung carcinoma epithelial cell), whole cell lysate at 20 µg

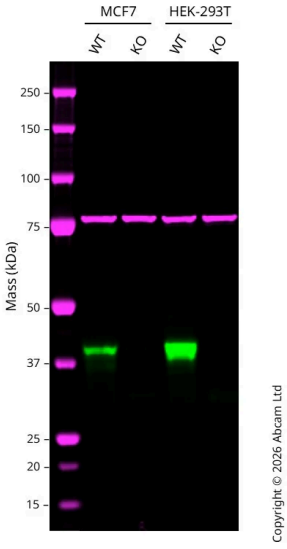
Lane 5:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 36 kDa

Observed band size: 42 kDa



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Western blot - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

Bmi1 Western blot staining using rabbit Anti-Bmi1 antibody

Western blot: Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade ab254253 staining at 1/5000 dilution, shown in green; Mouse anti-CANX ab238078 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 39 kDa in Wild-type MCF7 cell line with no signal observed at this size in BMI1 knockout MCF7 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253) at 1/5000 dilution

Lane 1:
Wild-type MCF7 at 20 µg

Lane 2:
Western blot - Human BMI1 knockout MCF7 cell line (ab262319) at 20 µg

Lane 3:
Wild-type HEK-293T at 20 µg

Lane 4:
BMI1 knockout HEK-293T at 20 µg

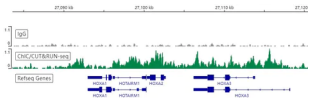
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 39 kDa



ChIC/CUT&RUN sequencing - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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 5µg of ab254253 [EPR22604-160]. 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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.