abcam

ab213723

Anti-Bmi1 (phospho T275) antibody [EPR19848]

Recombinant | RabMAb | 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	EPR19848
Purification technique	Affinity purification Protein A
Concentration	0.471 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Expected

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse

Dilution info	1/30
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	1/1000
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Species	Mouse
Dilution info	1/100
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Expected

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information Bmi1 pT275

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. The complex composed of RNF2, UB2D3 and BMI1 binds nucleosomes, and has activity only with nucleosomal histone H2A. In the PRC1-like complex, regulates the E3 ubiquitin-protein ligase activity of RNF2/RING2 (By similarity).

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.

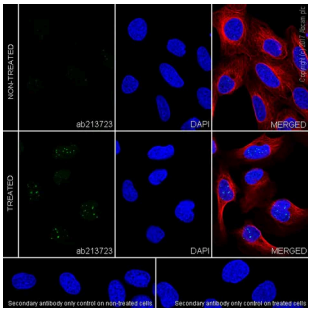
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

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6 product images

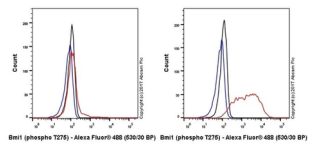


Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Bmi1 (phospho T275) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bmi1 (phospho T275) antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized U-2 OS (human bone osteosarcoma epithelial cell line) cells labeling Bmi1 (phospho T275) with ab213723 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing increased nuclear foci staining observed in UV-treated U-2 OS cells. The cells were treated with 50 J/m² UV, then cultured in McCoy's 5a media supplemented with 10% FBS for 2 hours.
The nuclear counterstain is DAPI (blue). Tubulin is detected with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (red).

The negative controls are as follows:
-ve control 1: PBS instead of primary antibody (non-treated cells), followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.
-ve control 2: PBS instead of primary antibody (UV treated cells), followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

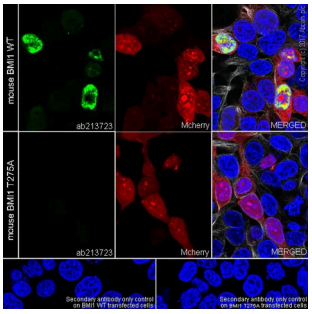
Bmi1 (phospho T275) Flow Cytometry (Intracellular) staining using rabbit Anti-Bmi1 (phospho T275) antibody

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell line transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector (left), or DDDDK and mCherry-tagged mouse Bmi1 WT expression vector (right) labeling Bmi1 (phospho T275) with ab213723 at 1/500 (red) compared with a Rabbit monoclonal IgG (ab172730)

(black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (black).
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077),at 1/2000 dilution was used as the secondary antibody.

The cells were gated on the mCherry positive population.

The plasmids were kindly provided by Dr. Wei Guo from Tsinghua University.



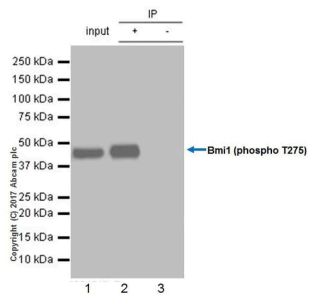
Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Bmi1 (phospho T275) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bmi1 (phospho T275) antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with DDDDK and mCherry-tagged mouse Bmi1 (WT) expression vector labeling Bmi1 (phospho T275) with ab213723 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 (WT) expression vector. No staining was observed in HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector.
The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (Alexa Fluor® 647) (ab195884) at 1/200 dilution (white).

The negative controls are as follows:
-ve control 1: PBS instead of primary antibody (HEK-293T transfected with wild-type BMi1), followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.
-ve control 2: PBS instead of primary antibody (HEK-293T transfected with Bmi1 T275A construct), followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

Plasmids were kindly provided by Dr. Wei Guo from Tsinghua University.



Immunoprecipitation - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Bmi1 (phospho T275) was immunoprecipitated from 0.35 mg of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) (transfected with DDDDK and mCherry-tagged mouse Bmi1 (WT) expression vector, followed by treatment with 800 J/m^2 UV, then cultured in DMEM containing 10% FBS for 30 minutes) whole cell lysate with ab213723 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab213723 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.
Lane 1: HEK-293T (transfected / UV treated) whole cell lysate 10 µg (Input).

Lane 2: ab213723 IP in HEK-293T (transfected / UV treated) lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab213723 in HEK-293T (transfected / UV treated) whole cell lysate.

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

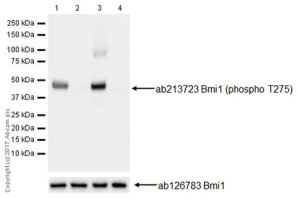
Exposure time: 10 seconds.

All lanes:
Immunoprecipitation - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Developed using the ECL technique.

Predicted band size: 36 kDa

Observed band size: 43 kDa



Western blot - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Blocking and dilution buffer: 2% BSA/TBST
The phosphorylation of Bmi1 at T275 is increased by UV treatment.

The expression plasmids were kindly provided by Dr. Wei Guo from Tsinghua University.

All lanes:
Western blot - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723) at 1/2000 dilution

Lane 1:
HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with DDDDK and mCherry-tagged mouse Bmi1 (WT) expression vector, whole cell lysate at 20 µg

Lane 2:
HEK-293T transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector, whole cell lysate at 20 µg

Lane 3:
HEK-293T transfected with DDDDK and mCherry-tagged mouse Bmi1 (WT) expression vector, followed by treatment with 800 J/m^2 UV, then cultured in DMEM containing 10% FBS for 30 minutes, whole cell lysate at 20 µg

Lane 4:
HEK-293T transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector, followed by treatment with 800 J/m^2 UV, then cultured in DMEM containing 10% FBS for 30 minutes, whole cell lysate at 20 µg

Secondary

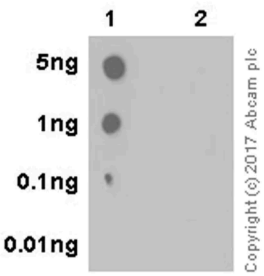
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 36 kDa

Observed band size: 43 kDa

Exposure time: 3s



Dot Blot - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

Dot blot analysis of Bmi1 (phospho T275) labeled with ab213723 at 1/1,000 dilution.
Lane 1: Bmi1 (phospho T275) peptide.

Lane 2: Bmi1 non-phospho peptide.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100,000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

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