abcam

Product datasheet

Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free ab228458

重组 RabMAb

6 图**像**

概述	
产品名称	Anti-Bmi1 (phospho T275) 抗体 [EPR19848] - BSA and Azide free
描述	兔单克隆抗体[EPR19848] to Bmi1 (phospho T275) - BSA and Azide free
宿主	Rabbit
经测试应 用	适用于: Flow Cyt (Intra), WB, Dot blot, ICC/IF, IP
种属反 应性	与反应: Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性 对照	ICC/IF: HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 (exposed to UV light).
常 规说 明	ab228458 is the carrier-free version of ab213723.
	Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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 <u>see here</u>.
	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. Do Not Freeze. 存储溶液 pH: 7.2 Constituent: PBS 无载体 是 纯**度** Protein A purified 克隆 单**克隆 克隆**编号 EPR19848 同种型 lgG

应用

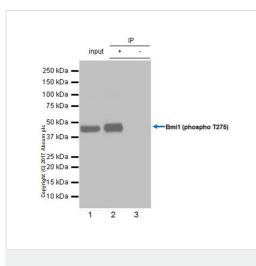
The Abpromise guarantee

Abpromise™承诺保证使用ab228458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)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 37 kDa.
Dot blot		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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.



Immunoprecipitation - Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free (ab228458) 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/m² 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 1/10000 dilution.

Lane 1: HEK-293T (transfected / UV treated) whole cell lysate 10 µg (Input).

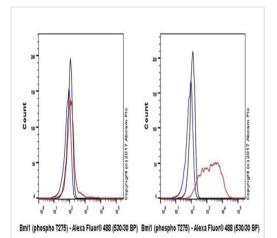
Lane 2: <u>ab213723</u> IP in HEK-293T (transfected / UV treated) lysate.

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

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

Exposure time: 10 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab213723</u>).



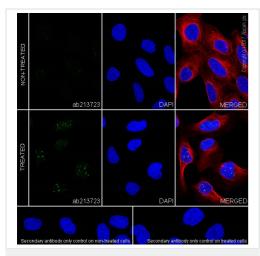
Flow Cytometry (Intracellular) - Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free (ab228458) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell 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 lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>), 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213723**).



Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free (ab228458)

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 <u>**ab195889**</u> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab213723</u>).

Dot blot analysis of Bmi1 (phospho T275) labeled with <u>ab213723</u> at 1/1,000 dilution.

Lane 1: Bmi1 (phospho T275) peptide.

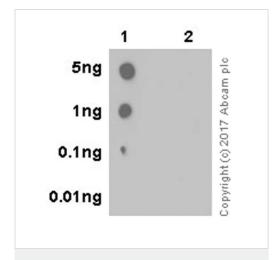
Lane 2: Bmi1 non-phospho peptide.

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

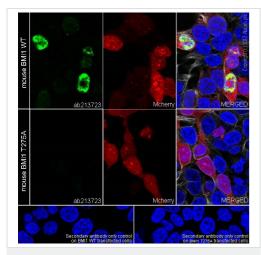
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab213723</u>).



Dot Blot - Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free (ab228458)



Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 (phospho T275) antibody [EPR19848] - BSA and Azide free (ab228458)

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 <u>ab213723</u> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) 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) (<u>ab195884</u>) 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) (<u>ab150077</u>) 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) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab213723</u>).

Why choose α recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant reproducible results technology Success from the Ethical standards first experiment compliant Animal-free Confirmed specificity production

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

BSA and Azide free (ab228458)

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