abcam

Product datasheet

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

リコンビナント RabMAb

画像数 7

製品の概要

製品名	Anti-Bmi1 (phospho T275) antibody [EPR19848]
製品の詳細	Rabbit monoclonal [EPR19848] to Bmi1 (phospho T275)
由来種	Rabbit
アプリケーション	**適用あり:** WB, Dot blot, ICC/IF, IP, Flow Cyt (Intra)
種交差性	**交差種:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 (+/- exposure to UV light). Dot blot: Bmi1 (phospho T275) peptide. ICC/IF: HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 (exposed to UV light) and U2OS cells. Flow Cyt (intra): HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1. IP: HEK-293T cells transfected with DDDDK and mCherry-tagged mouse Bmi1 (exposed to UV light).
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EPR19848
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab213723の使用に適用されます

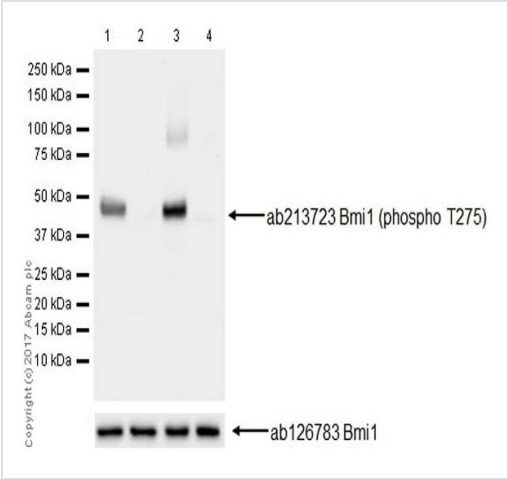
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/2000. Predicted molecular weight: 37 kDa.
Dot blot		1/1000.
ICC/IF		1/100.
IP		1/30.
Flow Cyt (Intra)		1/500.

ターゲット情報

機能	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.
配列類似性	Contains 1 RING-type zinc finger.
翻訳後修飾	Monoubiquitinated (By similarity). May be polyubiquitinated; which does not lead to proteasomal degradation.
細胞内局在	Nucleus. Cytoplasm.

画像



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

All lanes : 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
Lane 2 : HEK-293T transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector, whole cell lysate
Lane 3 : HEK-293T 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
Lane 4 : HEK-293T transfected with DDDDK and mCherry-tagged mouse Bmi1 T275A mutant expression vector, followed by treatment with 800 J/m² UV, then cultured in DMEM containing 10% FBS for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Developed using the ECL technique.

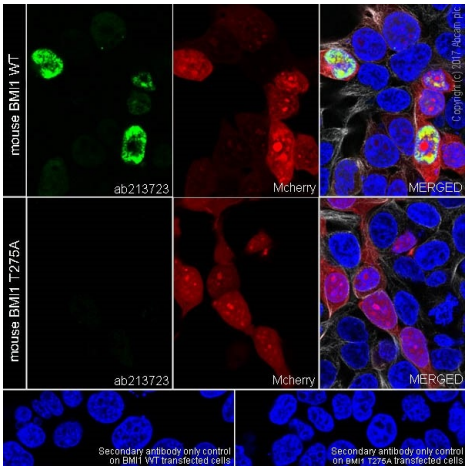
Predicted band size: 37 kDa
Observed band size: 43 kDa

Exposure time: 3 seconds

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.



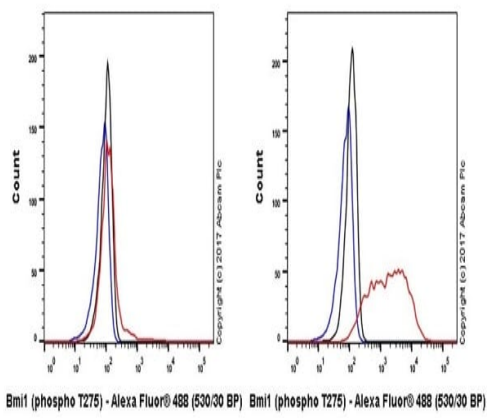
Immunocytochemistry/ Immunofluorescence - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

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.



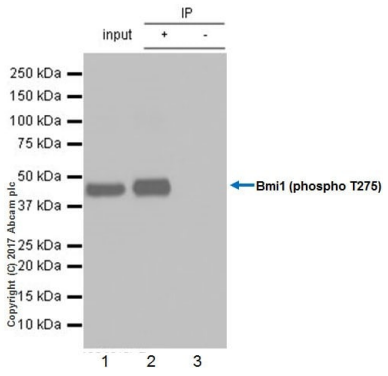
Flow Cytometry (Intracellular) - Anti-Bmi1 (phospho T275) antibody [EPR19848] (ab213723)

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.



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/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 1/10,000 dilution.

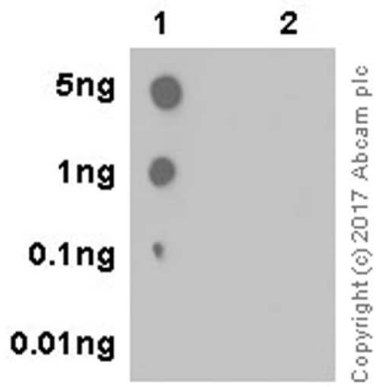
Lane 1: HEK-293T (transfected / UV treated) whole cell lysate 10 µg (Input).

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.



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

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

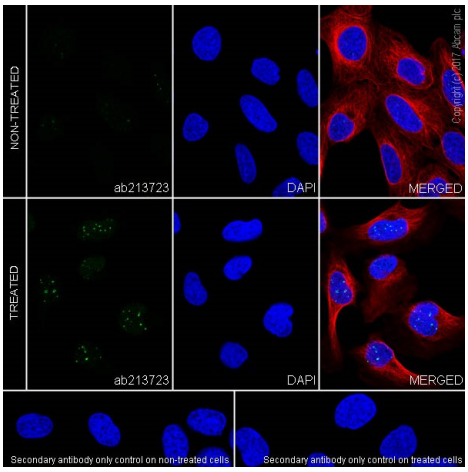
Lane 1: Bmi1 (phospho T275) peptide.

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.



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

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.

Anti-Bmi1 (phospho T275) antibody [EPR19848]

(ab213723)

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