abcam

Product datasheet

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

リコンビナント RabMAb

画像数 13

製品の概要

製品名	Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR22604-160] to Bmi1 - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, IP, ChIP, ICC/IF, IHC-P, ChIP-sequencing, WB
種交差性	**交差種:** Mouse, Human
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: K562, HeLa, MOLT-4, A549 and NIH/3T3 whole cell lysates IHC-P: Human gastric cancer, prostatic hyperplasia; Mouse colon ICC/IF: HeLa and NIH/3T3 cell lines Flow Cyt (intra): HeLa and NIH/3T3 cell lines IP: K562 cell lysate. ChIP seq : NCCIT cells. ChIC/CUT&RUN-Seq: NCCIT cells.
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	EPR22604-160

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ab254253の使用に適用されます

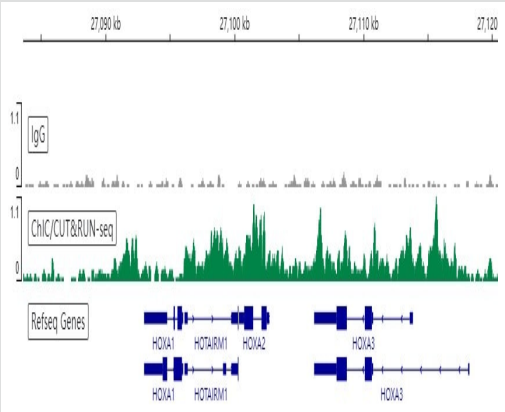
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/60.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
IP		1/30.
ChIP		Use at an assay dependent concentration. 5 µg
ICC/IF		1/50.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP-sequencing		Use 8µg for 10^7 cells.
WB		1/5000. Detects a band of approximately 42 kDa (predicted molecular weight: 36 kDa).

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

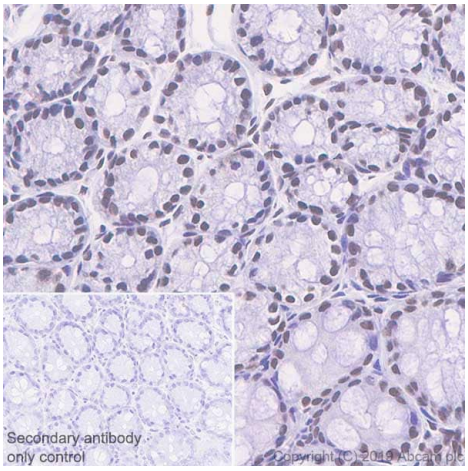


ChIC/CUT&RUN sequencing - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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 downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



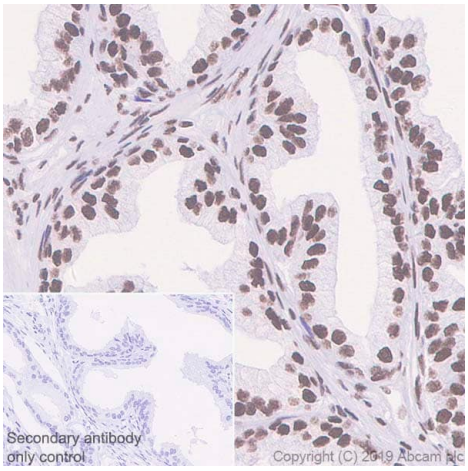
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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.



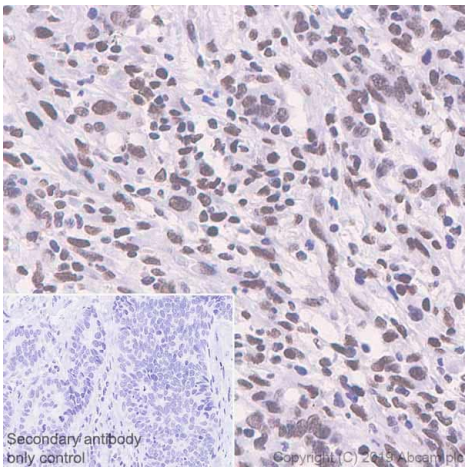
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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.



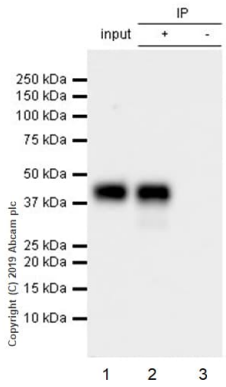
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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.



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.

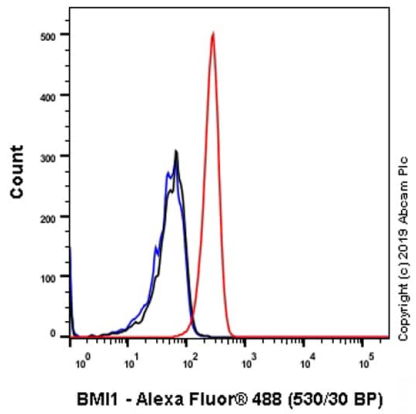
Lane 1: K562 whole cell lysate 10 µg (Input).

Lane 2: ab254253 IP in K562 whole cell lysate.

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

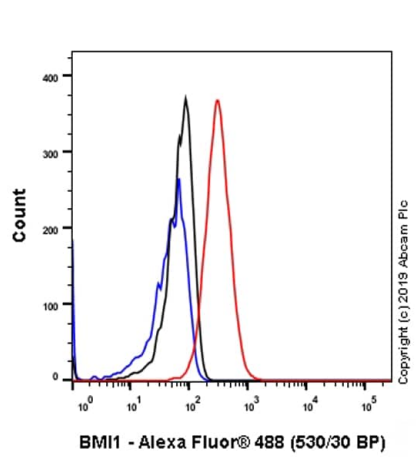
Blocking and dilution buffer and concentration: 5% NFDM/TBST

Exposure time: 15 seconds.



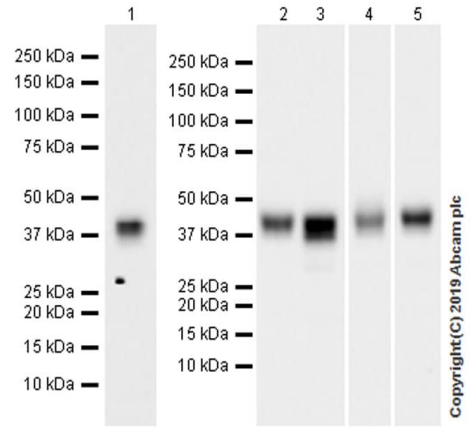
Flow Cytometry (Intracellular) - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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.



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

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.



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

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

Lane 1 : K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 3 : MOLT-4 (human lymphoblastic leukemia T lymphoblast), whole cell lysate
Lane 4 : A549 (human lung carcinoma epithelial cell), whole cell lysate
Lane 5 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

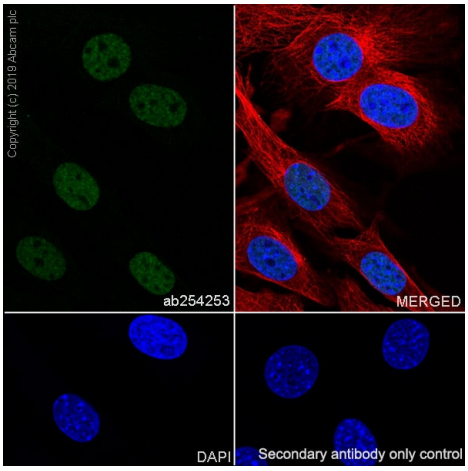
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 36 kDa
Observed band size: 42 kDa

Exposure time:
Lane 1: 48 seconds
Lanes 2-5: 10 seconds

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

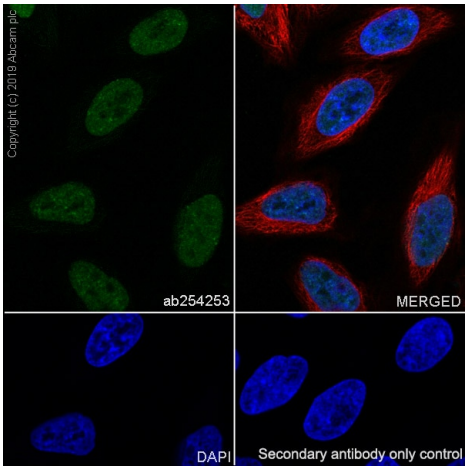
Molecular weight observed is consistent with what has been described in the literature (PMID: 26110620)



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

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.

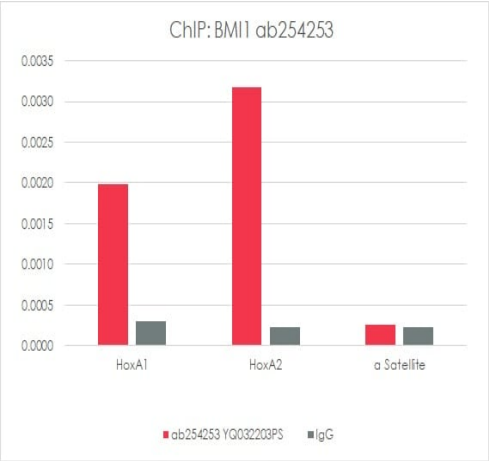
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a AlexaFluor®488 Goat anti-Rabbit (**ab150077**) secondary antibody at 1/1000 dilution.



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

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.

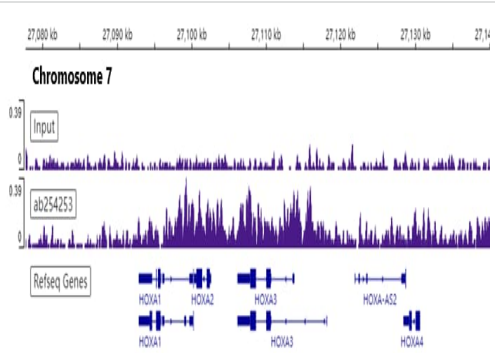


ChIP - Anti-Bmi1 antibody [EPR22604-160] - ChIP Grade (ab254253)

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**.

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

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