abcam

Product datasheet

Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade ab175187

KO 評価済 リコンビナント RabMAb

1 Abreviews 5 References 画像数 12

製品の概要

製品名	Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR5234(N)] to SUZ12 - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ChIP, ChIC/CUT&RUN-seq, WB, ICC/IF, IP **適用なし:** IHC-P
種交差性	**交差種:** Mouse, Human **交差が予測される動物種:** Rat
免疫原	Synthetic peptide within Human SUZ12 aa 50-150 (Cysteine residue). The exact sequence is proprietary. Database link: **Q15022**
ポジティブ・コントロール	WB: HAP1, Caco2, MCF7, SW480 and 293T cell lysate. IP: HeLa whole cell lysate. ChIP: HeLa and F9 cells. ICC/IF: MCF7 cells. Flow Cyt (intra): HeLa cells. ChIC/CUT&RUN-Seq: HeLa 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: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5234(N)
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ab175187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)		1/2000.
ChIP	★☆☆☆☆ (1)	Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
WB		1/1000 - 1/10000. Predicted molecular weight: 83 kDa.
ICC/IF		1/50 - 1/100.
IP		1/10 - 1/100.

追加情報 Is unsuitable for IHC-P.

ターゲット情報

機能 Polycomb group (PcG) protein. Component of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' (H3K9me) and 'Lys-27' (H3K27me) of histone H3, leading to transcriptional repression of the affected target gene. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1 and CDKN2A.

組織特異性 Overexpressed in breast and colon cancer.

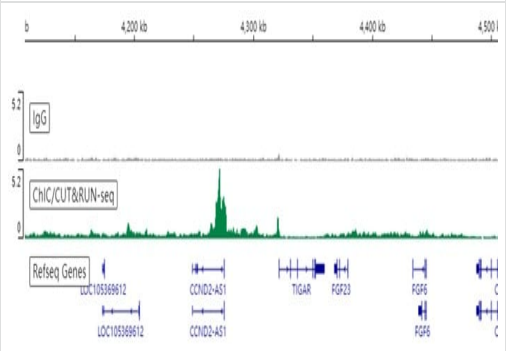
関連疾患 Note=A chromosomal aberration involving SUZ12 may be a cause of endometrial stromal tumors. Translocation t(7;17)(p15;q21) with JAZF1. The translocation generates the JAZF1-SUZ12 oncogene consisting of the N-terminus part of JAZF1 and the C-terminus part of SUZ12. It is frequently found in all cases of endometrial stromal tumors, except in endometrial stromal sarcomas, where it is rarer.

配列類似性 Belongs to the VEFS (VRN2-EMF2-FIS2-SU(Z)12) family.
Contains 1 C2H2-type zinc finger.

発生段階 Expressed at low levels in quiescent cells. Expression rises at the G1/S phase transition.

細胞内局在 Nucleus.

画像

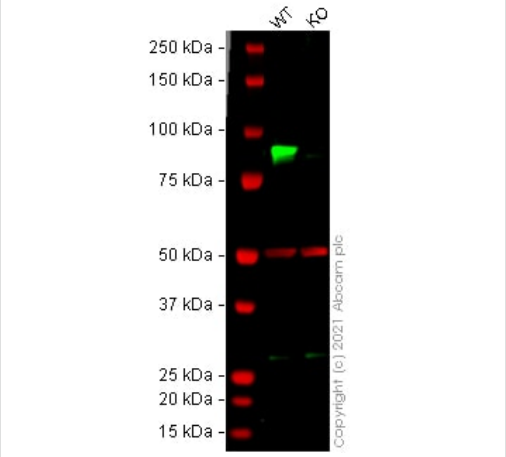


ChIC/CUT&RUN sequencing - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5µg of ab175187 [EPR5234(N)]. 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.



Western blot - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

All lanes : Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : SUZ12 knockout HAP1 cell lysate

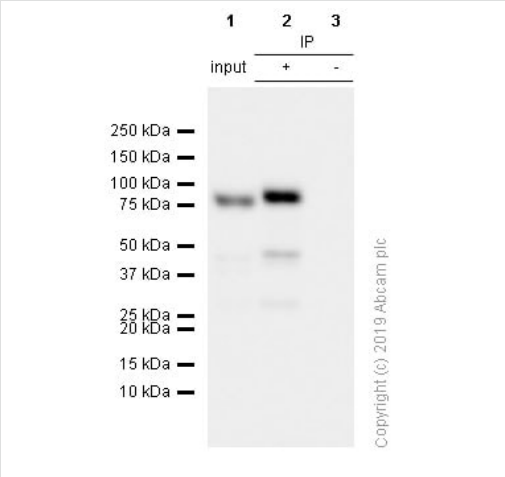
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 90 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab175187 observed at 90 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab175187 was shown to react with SUZ12 in wild-type HAP1 cells in Western blot with loss of signal observed in SUZ12 knockout sample. Wild-type HAP1 and SUZ12 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab175187 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



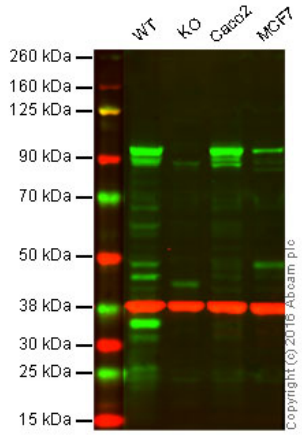
Immunoprecipitation - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

ab175187 (purified) at 1/20 dilution (16 µg/mL) immunoprecipitating SUZ12 in HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg.
Lane 1 (input): HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg
Lane 2 (+): ab175187 & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab175187 in HeLa whole cell lysate
For western blotting, ab175187 at 1/500 dilution (0.636 µg/mL) and veriBlot for IP secondary antibody (HRP) (**ab131366**) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.

ChIP - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

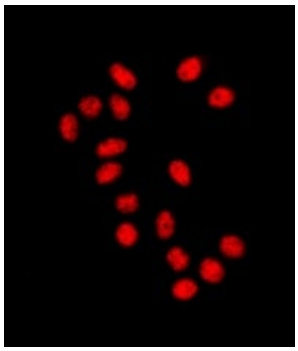
Chromatin was prepared from HeLa cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab175187 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG 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.
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Western blot - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

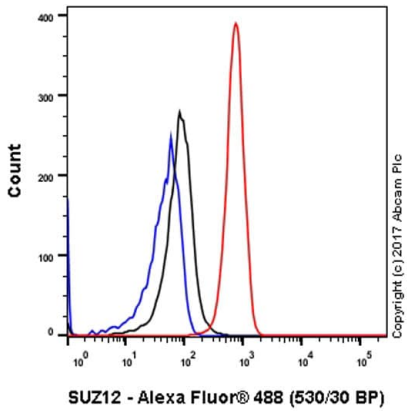
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SUZ12 knockout HAP1 cell lysate (20 µg)
Lane 3: Caco2 cell lysate (20 µg)
Lane 4: MCF7 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab175187 observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab175187 was shown to specifically react with SUZ12 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when SUZ12 knockout samples were used. Wild-type and SUZ12 knockout samples were subjected to SDS-PAGE. ab175187 and **ab8245** (loading control to GAPDH) were both 1/1000 and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1hr at room temperature before imaging.



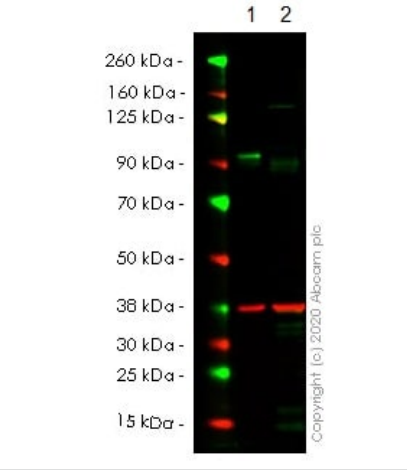
Immunocytochemistry/ Immunofluorescence - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

Immunofluorescence analysis of MCF-7 cells labeling SUZ12 with ab175187 at a 1/50 dilution.



Flow Cytometry (Intracellular) - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SUZ12 (red) with purified ab175187 at a 1/2000 dilution (1ug/mL). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (**ab172730**). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

All lanes : Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SUZ12 CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

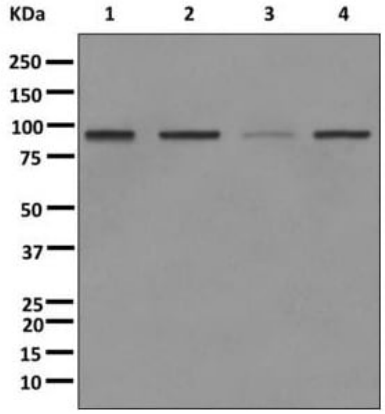
Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 100 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab175187 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab175187 was shown to react with SUZ12 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab264983** (CRISPR/Cas9 edited cell lysate **ab257721**) lane below 100kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and SUZ12 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab175187 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L

(IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



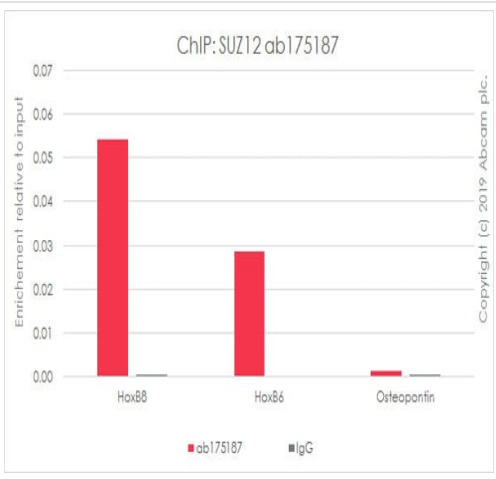
Western blot - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

All lanes : Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187) at 1/1000 dilution

Lane 1 : SW480 cell lysates
Lane 2 : HeLa cell lysates
Lane 3 : MCF-7 cell lysates
Lane 4 : 293T cell lysates

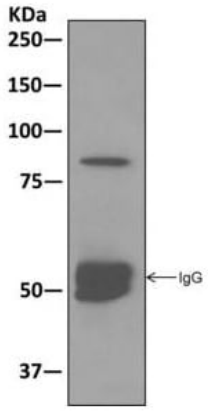
Lysates/proteins at 10 µg per lane.

Predicted band size: 83 kDa



ChIP - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

Chromatin was prepared from F9 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5 µg of ab175187 (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 (Taqman approach for active and inactive loci).
Primers and probes are located in the first kb of the transcribed region.



Immunoprecipitation - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

Western blot analysis on immunoprecipitation pellet from HeLa cell lysate using ab175187 at a 1/10 dilution.

Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

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