abcam

ab249844

Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR5234(N)
Purification technique	Affinity purification Protein A
Concentration	1.033 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-

Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

ICC/IF

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information SUZ12

Function	Polycomb group (PcG) protein. Component of the PRC2 complex, which methylates 'Lys-9' (H3K9me) and 'Lys-27' (H3K27me) of histone H3, leading to transcriptional repression of the affected target gene (PubMed:15225548, PubMed:15231737, PubMed:15385962, PubMed:16618801, PubMed:17344414, PubMed:18285464, PubMed:28229514, PubMed:29499137, PubMed:31959557). The PRC2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems (PubMed:12351676, PubMed:12435631, PubMed:15099518, PubMed:15225548, PubMed:15385962, PubMed:15684044, PubMed:16431907, PubMed:18086877, PubMed:18285464). Genes repressed by the PRC2 complex include HOXC8, HOXA9, MYT1 and CDKN2A (PubMed:15231737, PubMed:16618801, PubMed:17200670, PubMed:31959557).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab249844 is the carrier-free version of ab175187.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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 conjugation kits 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.

Compatibility
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.

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

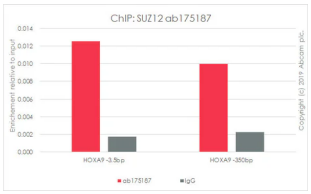
We do not recommend this combination. It is not covered by our product promise.

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.

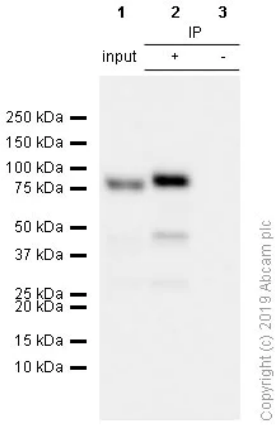
Full details and terms and conditions can be found here:
Terms & Conditions.

8 product images



ChIP - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab175187).

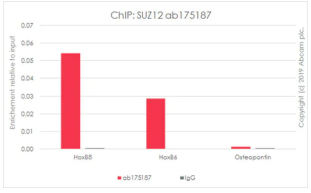


Immunoprecipitation - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

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.

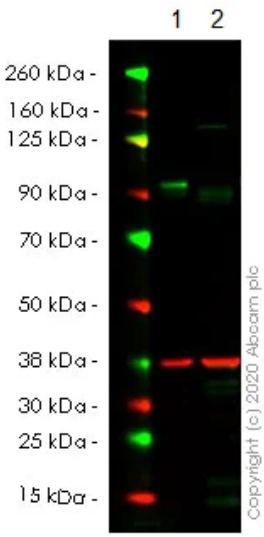
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab175187).
All lanes:
Immunoprecipitation - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187)

Predicted band size: 83 kDa



ChIP - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab175187).



Western blot - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

SUZ12 Western blot staining using rabbit Anti-SUZ12 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab175187).
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.

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

Lane 1:
Wild-type HeLa cell lysate at 20 µg

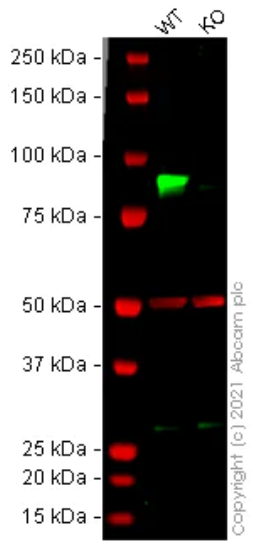
Lane 2:
SUZ12 CRISPR/Cas9 edited HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human SUZ12 knockout HeLa cell line (ab264983)

Performed under reducing conditions.

Predicted band size: 83 kDa

Observed band size: 100 kDa



Western blot - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

SUZ12 Western blot staining using rabbit Anti-SUZ12 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab175187).
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.

All lanes:

Western blot - Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (ab175187) at 1/1000 dilution

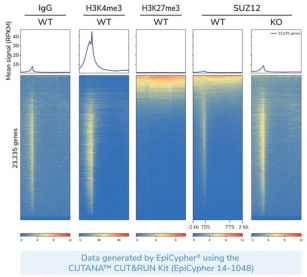
Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
SUZ12 knockout HAP1 cell lysate at 20 µg

Performed under reducing conditions.

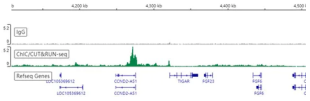
Predicted band size: 83 kDa

Observed band size: 90 kDa



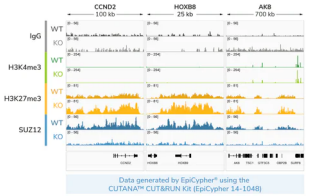
ChIC/CUT&RUN sequencing - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

This data was developed using ab175187, the same antibody clone in a different buffer formulation.
CUT&RUN profiling with SUZ12 antibody demonstrates robust genome-wide enrichment in wild-type (WT) cells, which is markedly diminished in SUZ12 knockout (KO) cells. Heatmaps of genome-wide signal flanking annotated gene bodies (transcription start sites [TSS - 2kb] to transcription termination sites [TTS + 2 kb]) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with SUZ12 antibody (Abcam ab175187, 0.5 µg). 500,000 HeLa WT (Abcam ab255448) or KO (Abcam ab264983) cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. H3K4me3 and H3K27me3 antibodies were included as positive controls, showing enrichment at regions of active transcription or repressive chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramírez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to SUZ12 WT, with red indicating high localized enrichment and blue denoting background. Top panels show mean reads per kilobase per million mapped reads (RPKM) for each target. Validated antibodies show genome-wide enrichment above IgG background consistent with SUZ12 binding in WT cells and near complete loss of signal in KO cells.



ChIC/CUT&RUN sequencing - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods. This data was developed using the same antibody clone in a different buffer formulation (ab175187).



ChIC/CUT&RUN sequencing - Anti-SUZ12 antibody [EPR5234(N)] - BSA and Azide free (ab249844)

This data was developed using ab175187, the same antibody clone in a different buffer formulation.

CUT&RUN profiling with SUZ12 antibody reveals the expected genomic enrichment pattern in wild-type (WT) cells, which is substantially reduced in SUZ12 knockout (KO) cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with SUZ12 antibody (Abcam ab175187, 0.5 µg). 500,000 HeLa WT (Abcam ab255448) or KO (Abcam ab264983) cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).

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