abcam

ab242149

Anti-CBX4 antibody [EPR23053-7] - ChIP Grade

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR23053-7
Purification technique	Affinity purification Protein A
Specificity	The human recommendation is based on the WB results. We do not guarantee IHC-P for human.
Concentration	0.58 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30

Notes	-

Expected

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

ChIP

Tested

Species	Human
Dilution info	2 µg for 25 µg chromatin
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000

Notes	-

ICC/IF

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/600
Notes	-

Expected

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

IHC-P

Tested

Species	Mouse
Dilution info	1/200
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/200
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

IHC-Fr

Not recommended

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

Target data

See full target information CBX4

Function

E3 SUMO-protein ligase that catalyzes sumoylation of target proteins by promoting the transfer of SUMO from the E2 enzyme to the substrate (PubMed:12679040, PubMed:22467880, PubMed:22825850). Also acts as a histone reader, which specifically recognizes and binds histone H3 trimethylated at 'Lys-9' and 'Lys-27' (H3K9me3 and H3K27me3, respectively) via its chromo domain (By similarity). Catalyzes sumoylation of HNRNPK, a p53/TP53 transcriptional coactivator, hence indirectly regulates p53/TP53 transcriptional activation resulting in p21/CDKN1A expression (PubMed:22825850). Acts as a regulator of brown adipocyte differentiation by mediating sumoylation of PRDM16, thereby preventing PRDM16 ubiquitination and degradation (By similarity). Monosumoylates ZNF131 (PubMed:22467880). Component of a Polycomb group (PcG) multiprotein PRC1-like complex, a complex class required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development (PubMed:12167701, PubMed:19636380, PubMed:21282530). 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 (PubMed:12167701, PubMed:19636380, PubMed:21282530). Plays a role in the lineage differentiation of the germ layers in embryonic development (By similarity).

Storage

Shipped at conditions Conditional Ambient

Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CBX4 also known as Chromobox protein homolog 4 or Polycomb 2 acts mechanically as part of the Polycomb group (PcG) proteins. It weighs approximately 60 kDa and it is found in nuclear matrix where it participates in chromatin remodeling. CBX4 shows significant expression in tissues like liver brain and lung indicating its wide importance to cellular function. By binding to methylated histones especially H3K27me3 CBX4 influences gene silencing and epigenetic regulation important for maintaining cellular identity.
Biological function summary	CBX4 operates as a component of the Polycomb Repressive Complex 1 (PRC1) which plays role in controlling gene expression patterns during development. In this complex CBX4 interacts with other core components such as BMI1 and RING1 to facilitate monoubiquitination of histone H2A leading to transcriptional repression. This ability of CBX4 to modulate epigenetic states highlights its importance in critical developmental processes and differentiation.
Pathways	CBX4 significantly influences gene regulatory networks involved in cell cycle and apoptosis pathways. In the cell cycle regulation CBX4 interacts with important

	proteins such as Cyclin D1 impacting cell proliferation. In apoptosis CBX4 has roles linked to the p53 pathway by modulating transcriptional activity which influences cellular fate. These pathways underline the significance of CBX4 in maintaining balance between cell growth and death.
Associated diseases and disorders	The deregulation of CBX4 expression has been associated with cancers like hepatocellular carcinoma and breast cancer. CBX4 potentially interacts with proteins such as p21 and p16 whose pathways play significant roles in cancer development. This underlines its role as a potential biomarker or therapeutic target in cancer treatment strategies. Understanding the function and regulation of CBX4 could offer insights in devising novel treatments for these malignancies.

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

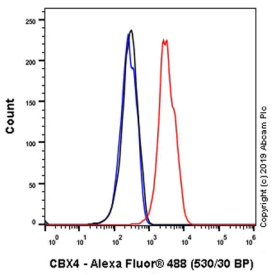
We do not recommend this combination. It is not covered by our product promise.

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.

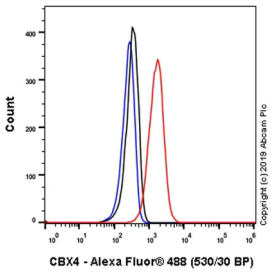
11 product images



Flow Cytometry (Intracellular) - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Flow Cytometry (Intracellular) staining using rabbit Anti-CBX4 antibody

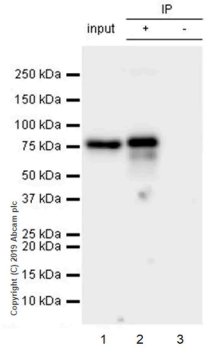
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HCT 116 (Human colorectal carcinoma epithelial cell) cells labelling CBX4 with ab242149 at 1/600 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Flow Cytometry (Intracellular) staining using rabbit Anti-CBX4 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized NCCIT (Human pluripotent embryonic carcinoma epithelial cell) cells labelling CBX4 with ab242149 at 1/600 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 was immunoprecipitated from 0.35 mg HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate with ab242149 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab242149 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate 10ug

Lane 2: ab242149 IP in HCT 116 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab242149 in HCT 116 whole cell lysate

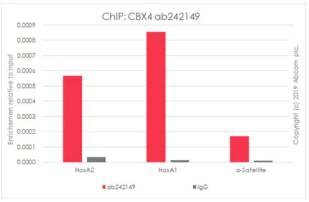
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds.

All lanes:
Immunoprecipitation - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

Predicted band size: 52 kDa, 61 kDa

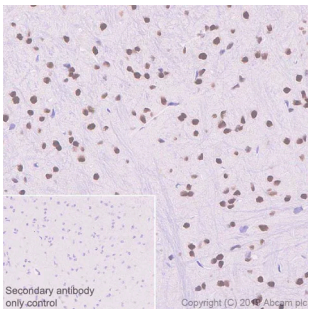
Observed band size: 45 kDa, 60 kDa, 66 kDa, 74 kDa



ChIP - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

Chromatin was prepared from human NCCIT cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with 1.5 mM EGS for 30mins and then 1% formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 2 µg of ab242149 (red), or 2 µg of rabbit normal IgG ab172730 (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CBX4 antibody

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling CBX4 with ab242149 at 1/200 dilution (2.785 ug/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Nuclear staining on mouse cerebrum (PMID: 28283560). The section was incubated with ab242149 for 30 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

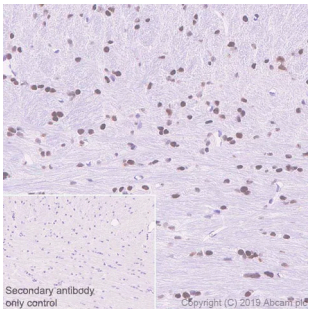


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CBX4 antibody

Immunohistochemical analysis of paraffin-embedded Mouse hippocampus tissue labeling CBX4 with ab242149 at 1/200 dilution (2.785 ug/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Nuclear staining on mouse hippocampus (PMID: 28283560). The section was incubated with ab242149 for 30 at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



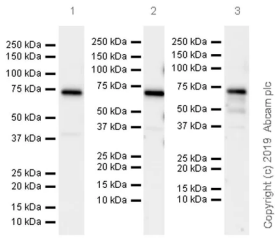
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CBX4 antibody

Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue labeling CBX4 with ab242149 at 1/200 dilution (2.785 ug/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Nuclear staining on rat hippocampus (PMID: 28283560). The section was incubated with

ab242149 for 30 at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Western blot staining using rabbit Anti-CBX4 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
Exposure time: 26 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 27864346).

All lanes:
Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149) at 1/1000 dilution

Lane 1:
HCT 116 (human colorectal carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate at 20 µg

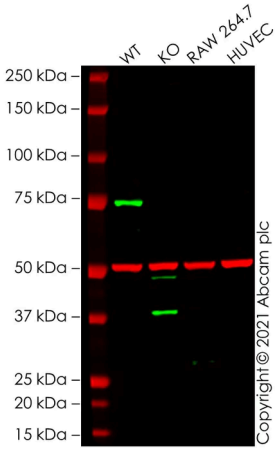
Lane 3:
C6 (rat glial tumor glial cell), whole cell lysate at 20 µg

Secondary

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

Predicted band size: 61 kDa

Observed band size: 74 kDa



Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Western blot staining using rabbit Anti-CBX4 antibody

False colour image of Western blot: Anti-CBX4 antibody [EPR23053-7] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab242149 was shown to bind specifically to CBX4. A band was observed at 75 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in Cbx4 knockout cell line ab261723 (knockout cell lysate ab261666). The band observed in the knockout lysate lane below 75 kDa is likely to represent a truncated form of CBX4. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Cbx4 knockout HEK-293 cell lysates were analysed.First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then

imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149) at 1/1000 dilution

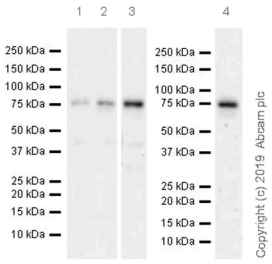
Lane 1:
Wild-type HEK-293 cell lysate at 20 µg

Lane 2:
Cbx4 knockout HEK-293 cell lysate at 20 µg

Lane 3:
RAW 264.7 cell lysate at 20 µg

Lane 4:
HUVEC cell lysate at 20 µg

Performed under reducing conditions.



Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Western blot staining using rabbit Anti-CBX4 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
Exposure time: 3 minutes.

The molecular weight observed is consistent with what has been described in the literature (PMID: 27864346).

All lanes:
Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149) at 1/1000 dilution

Lane 1:
HDLM-2 (human Hodgkin lymphoma) whole cell lysate at 20 µg

Lane 2:
NCCIT (human pluripotent embryonic carcinoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
Mouse thymus tissue lysate at 20 µg

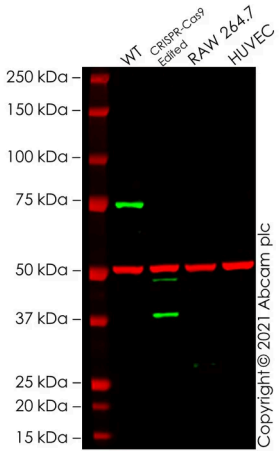
Lane 4:
Rat thymus tissue lysate at 20 µg

Secondary

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

Predicted band size: 61 kDa

Observed band size: 74 kDa



Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149)

CBX4 Western blot staining using rabbit Anti-CBX4 antibody

False colour image of Western blot: Anti-CBX4 antibody [EPR23053-7] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab242149 was shown to bind specifically to CBX4. A band was observed at 75 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in Cbx4 CRISPR-Cas9 edited cell line ab261723 (CRISPR-Cas9 edited cell lysate ab261666). The band observed in the CRISPR-Cas9 edited lysate lane below 75 kDa is likely to represent a truncated form of CBX4. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Cbx4 CRISPR-Cas9 edited HEK-293 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-CBX4 antibody [EPR23053-7] - ChIP Grade (ab242149) at 1/1000 dilution

Lane 1:
Wild-type HEK-293 cell lysate at 20 µg

Lane 2:
Cbx4 CRISPR-Cas9 edited HEK-293 cell lysate at 20 µg

Lane 3:
RAW 264.7 cell lysate at 20 µg

Lane 4:
HUVEC cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 61 kDa

Observed band size: 75 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.