

Product datasheet

Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free ab264077

Recombinant RabMAb

7 Images

Overview	
Product name	Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free
Description	Rabbit monoclonal [EPR22858-106] to RUNX2 - BSA and Azide free
Host species	Rabbit
Specificity	ab264077 is not recommended for mouse IHC.
Tested applications	Suitable for: ChIC/CUT&RUN-seq, WB, IP, IHC-P, ChIP Unsuitable for: Flow Cyt or ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: PC-3, MDA-MB-231, Saos-2, MEF, C2C12, C6 and PC-12 lysates. IHC-P: Human osteosarcoma and Rat embryo 14.5 day tissues. IP: PC-3 and Saos-2 cells. ChIC/CUT&RUN seq: Saos-2 cell
General notes	ab264077 is the carrier-free version of <u>ab236639</u> .
	Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.
	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.
	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.
	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
	- Animaritee 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 <u>RabMAb[®] patents</u>.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22858-106
lsotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab264077 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 57 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
ChIP		Use 5 µg for 25 µg of chromatin.

Application notes

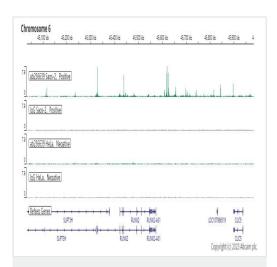
Is unsuitable for Flow Cyt or ICC/IF.

Target

Function	Transcription factor involved in osteoblastic differentiation and skeletal morphogenesis. Essential for the maturation of osteoblasts and both intramembranous and endochondral ossification. CBF binds to the core site, 5'-PYGPYGGT-3', of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, osteocalcin, osteopontin, bone sialoprotein, alpha 1(I) collagen, LCK, IL-3 and GM-CSF promoters (By similarity). Inhibits MYST4-dependent transcriptional activation.
Tissue specificity	Specifically expressed in osteoblasts.
Involvement in disease	Defects in RUNX2 are the cause of cleidocranial dysplasia (CLCD) [MIM:119600]; also known as

	cleidocranial dysostosis (CCD). CLCD is an autosomal dominant skeletal disorder with high
	penetrance and variable expressivity. It is due to defective endochondral and intramembranous
	bone formation. Typical features include hypoplasia/aplasia of clavicles, patent fontanelles,
	wormian bones (additional cranial plates caused by abnormal ossification of the calvaria),
	supernumerary teeth, short stature, and other skeletal changes. In some cases defects in RUNX2
	are exclusively associated with dental anomalies.
Sequence similarities	Contains 1 Runt domain.
Domain	A proline/serine/threonine rich region at the C-terminus is necessary for transcriptional activation
	of target genes and contains the phosphorylation sites.
Post-translational	Phosphorylated; probably by MAP kinases (MAPK) (By similarity). Isoform 3 is phosphorylated on
modifications	Ser-340.
Cellular localization	Nucleus.

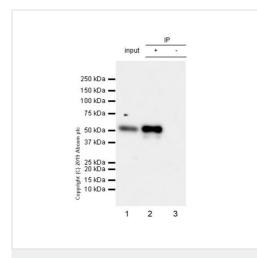
Images



ChIC/CUT&RUN sequencing - Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free (ab264077) This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/ μ L, 2.5X10^5 of positive cell line Saos-2 or low expression cell line HeLa were used along with 5 μ g of **ab236639** [EPR22858-106]. 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 <u>here</u>.

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



Immunoprecipitation - Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free (ab264077) RUNX2 was immunoprecipitated from 0.35 mg Saos-2 (human osteosarcoma epithelial) whole cell lysate with **ab236639** at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab236639** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

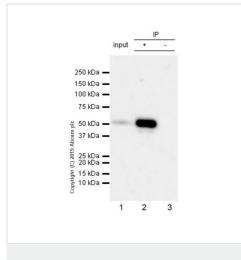
Lane 1: Saos-2 (human osteosarcoma epithelial) whole cell lysate 10ug

Lane 2: ab236639 IP in Saos-2 whole cell lysate

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of $\underline{ab236639}$ in Saos-2 whole cell lysate

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

Exposure time: 30 seconds





This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).

RUNX2 was immunoprecipitated from 0.35 mg PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate 10ug with <u>ab236639</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab236639</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate 10ug

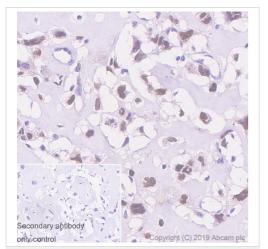
Lane 2: ab236639 IP in PC-3 whole cell lysate

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of $\underline{ab236639}$ in PC-3 whole cell lysate

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

Exposure time: 30 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).

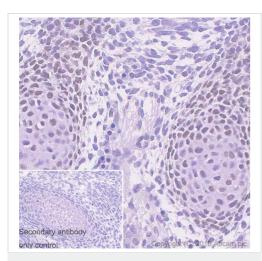


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free (ab264077) Immunohistochemical analysis of paraffin-embedded human osteosarcoma tissue labeling RUNX2 with <u>ab236639</u> at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on human osteosarcoma (PMID: 21731849) is observed. The section was incubated with <u>ab236639</u> for 15 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

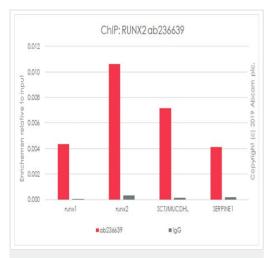
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free (ab264077)



ChIP - Anti-RUNX2 antibody [EPR22858-106] - BSA and Azide free (ab264077) Immunohistochemical analysis of paraffin-embedded rat embryo 14.5 day tissue labeling RUNX2 with <u>ab236639</u> at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on the cartilage cells in the rat embryo 14.5 day tissue is observed. The section was incubated with <u>ab236639</u> for 15 mins at room temperature. 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 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).

Chromatin was prepared from Saos-2 cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of **ab236639** (red), or 5 μ g of rabbit normal IgG **ab172730** (gray) and 20 μ I 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab236639</u>).



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