abcam

Product datasheet

Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade ab236639

Recombinant RabMAb

40 References 10 Images

Overview

Product name	Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade
Description	Rabbit monoclonal [EPR22858-106] to RUNX2 - ChIP Grade
Host species	Rabbit
Specificity	ab236639 is not recommended for mouse IHC.
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, IP, ChIP, IHC-P, WB **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	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**.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22858-106
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab236639 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.
IP		1/30.
ChIP		Use at an assay dependent concentration. Use at 5µg
IHC-P		1/2000.
WB		1/1000. Predicted molecular weight: 57 kDa.

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.

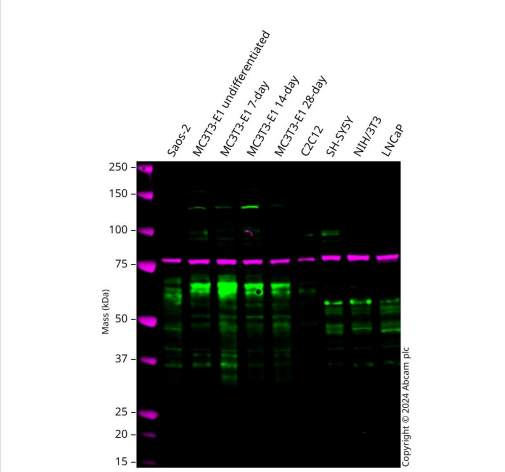
Sequence similarities Contains 1 Runt domain.

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 modifications Phosphorylated; probably by MAP kinases (MAPK) (By similarity). Isoform 3 is phosphorylated on Ser-340.

Cellular localization Nucleus.

Images



Western blot - Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639)

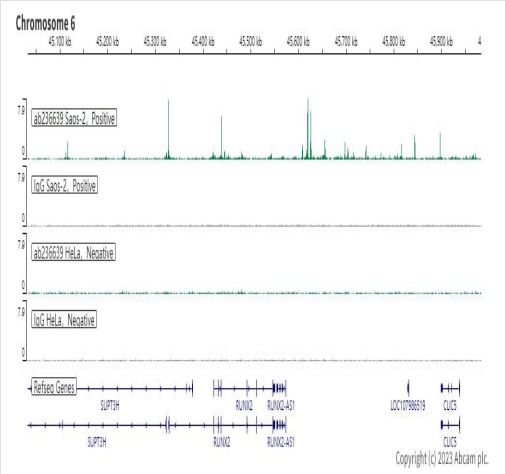
All lanes : Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639) at 1/1000 dilution

Lane 1 : Saos-2 cell lysate
Lane 2 : MC3T3-E1 undifferentiated cell lysate
Lane 3 : MC3T3-E1 7-day Osteogenic differentiation cell lysate
Lane 4 : MC3T3-E1 14-day Osteogenic differentiation cell lysate
Lane 5 : MC3T3-E1 28-day Osteogenic differentiation cell lysate
Lane 6 : C2C12 cell lysate
Lane 7 : SH-SY5Y cell lysate
Lane 8 : NIH/3T3 cell lysate
Lane 9 : LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

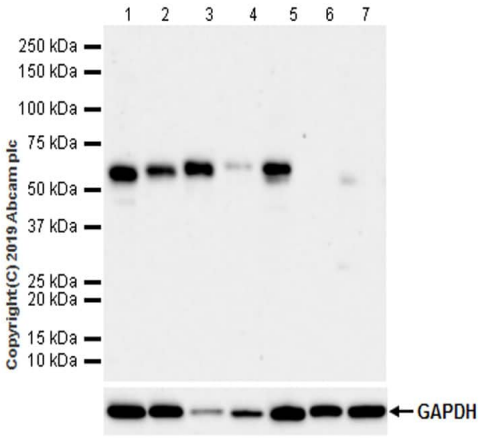
Predicted band size: 57 kDa
Observed band size: 60 kDa

Western blot: Anti-RUNX2 antibody [EPR22858-106] (ab236639) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab236639 was shown to bind specifically to RUNX2. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution



ChIC/CUT&RUN sequencing - Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL, 2.5X10^5 of positive cell line Saos-2 or low expression cell line HeLa were used along with 5µg 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 **here**.
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

All lanes : Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639) at 1/1000 dilution

Lane 1 : PC-3 (human prostate adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : MDA-MB-231 (human breast adenocarcinoma epithelial cell), whole cell lysate
Lane 3 : Saos-2 (human osteosarcoma epithelial), whole cell lysate
Lane 4 : MEF (mouse embryonic fibroblast (immortalized)), whole cell lysate
Lane 5 : C2C12 (mouse myoblasts myoblast), whole cell lysate
Lane 6 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate
Lane 7 : HeLa (human cervix adenocarcinoma epithelial cell), 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

Predicted band size: 57 kDa

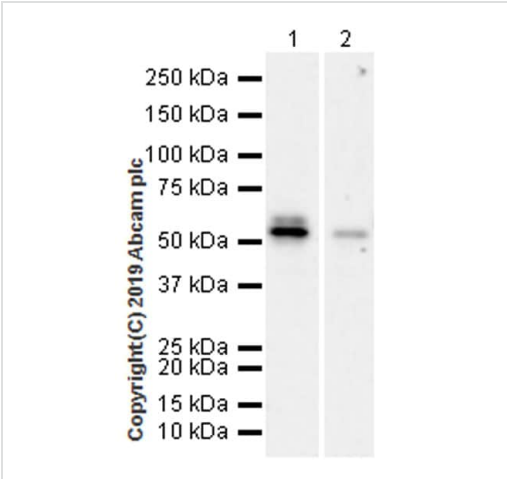
Observed band size: 60,64 kDa

Lysates were made freshly and used in WB test immediately to minimize protein degradation.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID:19419310)

Negative control: MCF-7 and HeLa (PMID: 20591170)

Exposure time: 70 seconds



Western blot - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

All lanes : Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639) at 1/1000 dilution

Lane 1 : C6 (rat glial tumor glial cell), whole cell lysate
Lane 2 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

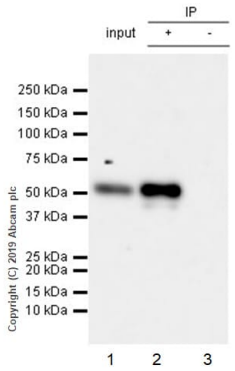
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 57 kDa
Observed band size: 60,64 kDa

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 19419310, 20591170)

Exposure time: 3 mintues



Immunoprecipitation - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

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.

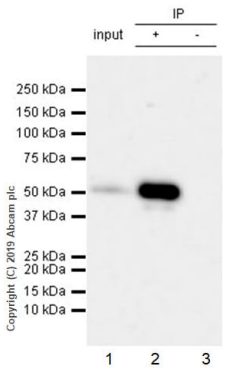
Lane 1: Saos-2 (human osteosarcoma epithelial) whole cell lysate 10ug

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

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab236639 in Saos-2 whole cell lysate

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

Exposure time: 30 seconds



Immunoprecipitation - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

RUNX2 was immunoprecipitated from 0.35 mg PC-3 (human prostate adenocarcinoma epithelial cell) 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.

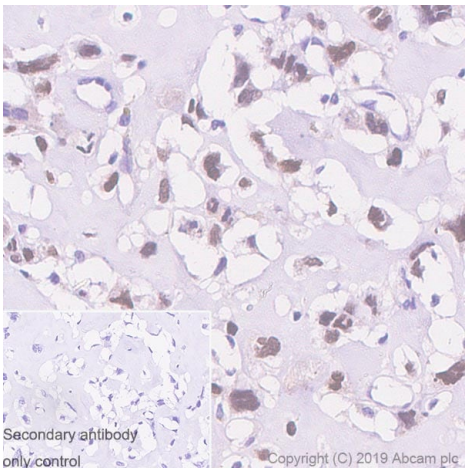
Lane 1: PC-3 whole cell lysate 10ug

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

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab236639 in PC-3 whole cell lysate

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

Exposure time: 30 seconds

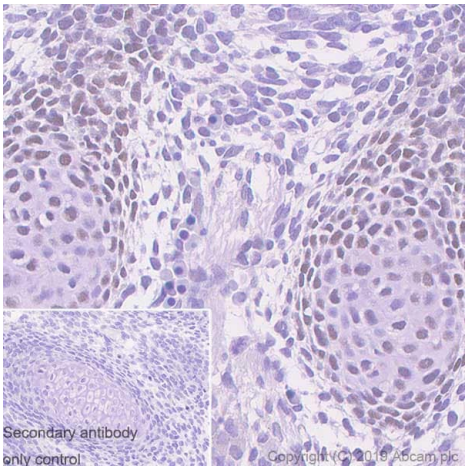


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

Immunohistochemical analysis of paraffin-embedded human osteosarcoma tissue labeling RUNX2 with ab236639 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human osteosarcoma (PMID: 21731849) is observed. The section was incubated with ab236639 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

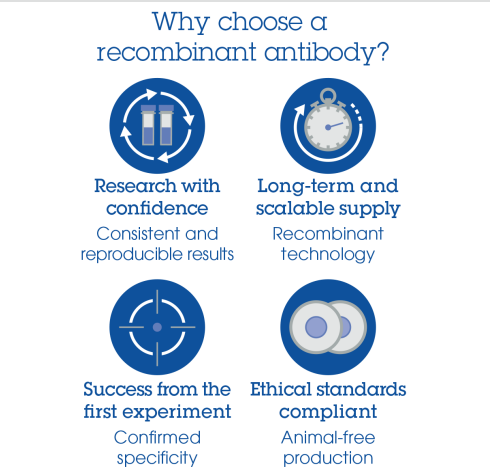
Immunohistochemical analysis of paraffin-embedded rat embryo 14.5 day tissue labeling RUNX2 with ab236639 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the cartilage cells in the rat embryo 14.5 day tissue. The section was incubated with ab236639 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.

ChIP - Anti-RUNX2 antibody [EPR22858-106] (ab236639)

Legend 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 µg of chromatin, 5 µg of ab236639 (red), or 5 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). Primers and probes are commercial primers from PMCID: PMC3281617



Anti-RUNX2 antibody [EPR22858-106] - ChIP Grade (ab236639)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors