abcam

Product datasheet

Anti-RUNX3 antibody [EPR20687] - ChIP Grade ab224641

KO VALIDATED Recombinant RabMAb

7 References 12 Images

Overview

Product name	Anti-RUNX3 antibody [EPR20687] - ChIP Grade
Description	Rabbit monoclonal [EPR20687] to RUNX3 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ChIP, Flow Cyt (Intra), WB, IP, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji and Ramos cell lysate; human tonsil tissue lysate. IHC: Human stomach, gastric cancer and diffuse large B cell lymphoma tissue. ICC/IF: Ramos and Raji cells. Flow Cyt (intra): Raji cells. IP: Raji cell lysate.
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, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR20687
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab224641 in the following tested applications.

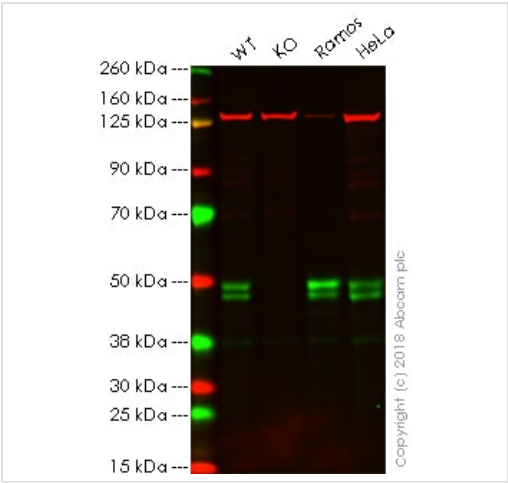
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP		Use at an assay dependent concentration.
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 44, 46 kDa (predicted molecular weight: 44 kDa).
IP		1/30.
ICC/IF		1/100.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function	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, lck, IL-3 and GM-CSF promoters.
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.
Post-translational modifications	Phosphorylated on tyrosine residues by SRC. Phosphorylated by LCK and FYN.
Cellular localization	Nucleus. Cytoplasm. The tyrosine phosphorylated form localizes to the cytoplasm.

Images



Western blot - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

All lanes : Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641) at 1/1000 dilution

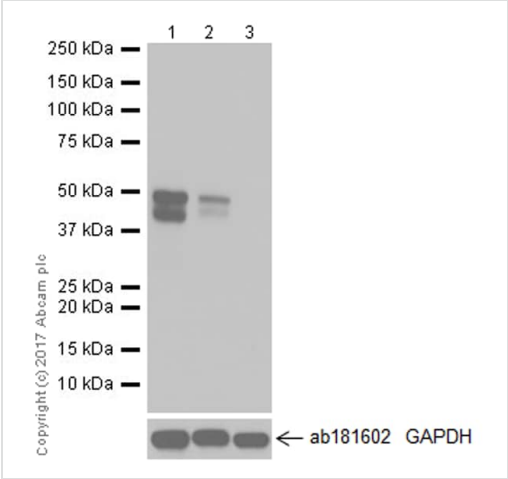
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : RUNX3 knockout HAP1 whole cell lysate
Lane 3 : Ramos whole cell lysate
Lane 4 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 44 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab224641 observed at 44-46 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab224641 was shown to specifically react with RUNX3 in wild-type HAP1 cells as signal was lost in RUNX3 knockout cells. Wild-type and RUNX3 knockout samples were subjected to SDS-PAGE. Ab224641 and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

All lanes : Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641) at 1/1000 dilution

Lane 1 : Raji (human Burkitt's lymphoma cell line) cell lysate
Lane 2 : Ramos (human Burkitt's lymphoma cell line) cell lysate
Lane 3 : MCF7 (human breast adenocarcinoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

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

Developed using the ECL technique.

Performed under reducing conditions.

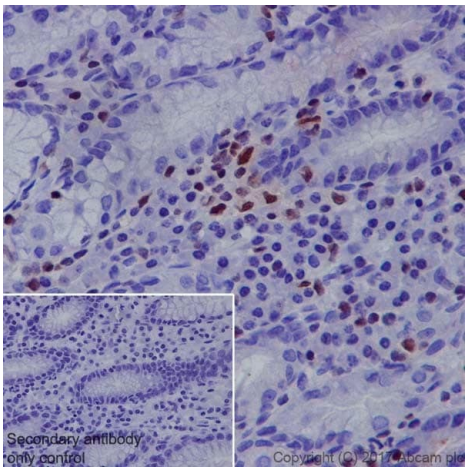
Predicted band size: 44 kDa
Observed band size: 44, 46 kDa

Exposure time: 5 seconds

Blocking/Dilution: 5% NFDM/TBST.

Negative control: MCF7 (PMID:21706051).

This target detects both predicted isoforms 44KDa and 46KDa that consistent with what has been described in the literature (PMID:23700080).

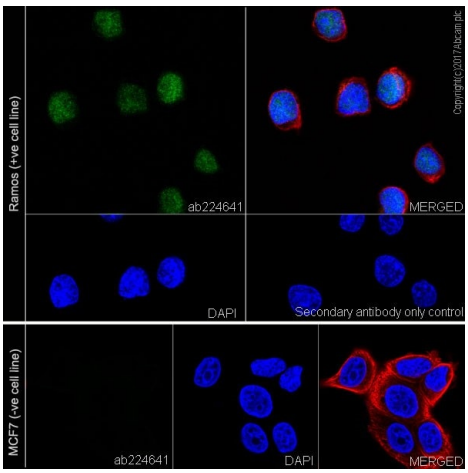


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling RUNX3 with ab224641 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use. Nuclear staining on lymphoid cells of human stomach (PMID:15514019; PMID:21786422) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use.

Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



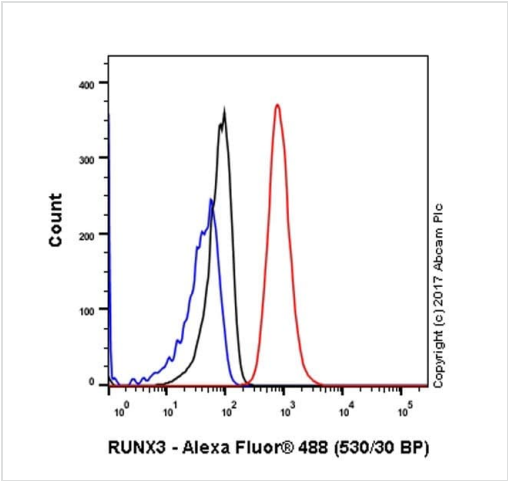
Immunocytochemistry/ Immunofluorescence - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Ramos (human Burkitt's lymphoma cell line) cells labeling RUNX3 with ab224641 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on Ramos cell line. DAPI (blue) and anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution were used as counterstains.

The negative controls are as follows:

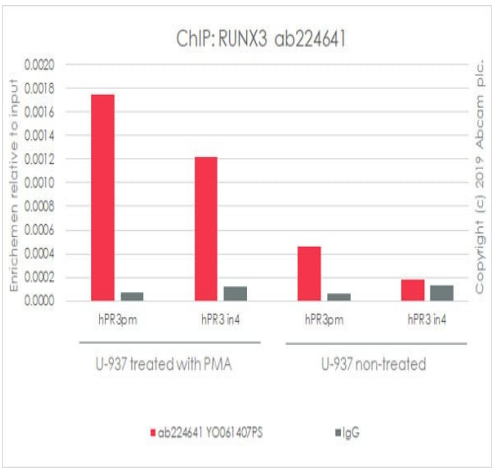
Negative control: MCF7 (human breast adenocarcinoma cell line) (PMID: 21706051).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit (**ab150077**) at 1/1000 dilution..



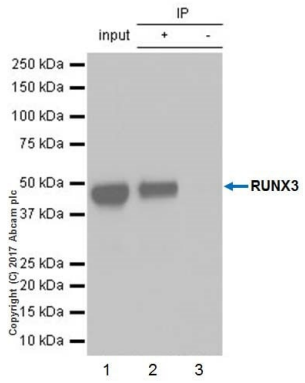
Flow Cytometry (Intracellular) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized Raji (human Burkitt's lymphoma cell line) cell line labeling RUNX3 with ab224641 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.



ChIP - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Chromatin was prepared from U-937 (PMA treated or not) cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab224641 (red), and 20µl of protein A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Immunoprecipitation - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

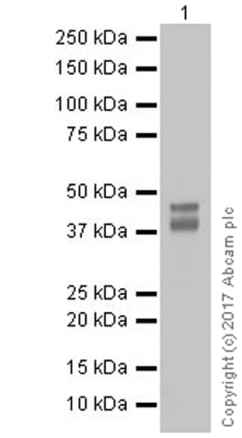
RUNX3 was immunoprecipitated from 0.35 mg of Raji (human Burkitt's lymphoma cell line) whole cell lysate with ab224641 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab224641 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Lane 1: Raji whole cell lysate 10 µg (Input).

Lane 2: ab224641 IP in Raji whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab224641 in Raji whole cell lysate (-).

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



Western blot - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641) at 1/1000 dilution + Human tonsil tissue lysate at 20 µg

Secondary

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

Developed using the ECL technique.

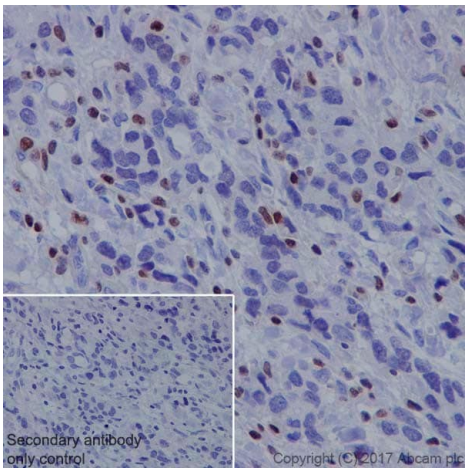
Performed under reducing conditions.

Predicted band size: 44 kDa

Exposure time: 30 seconds

Blocking/Dilution: 5% NFDM/TBST.

This target detects both predicted isoforms 44KDa and 46KDa that consistent with what has been described in the literature (PMID:23700080).

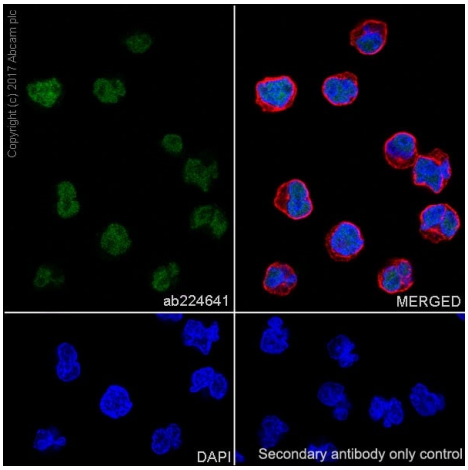


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling RUNX3 with ab224641 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use. Nuclear staining on lymphoid cells of human gastric cancer (PMID:27566570) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use.

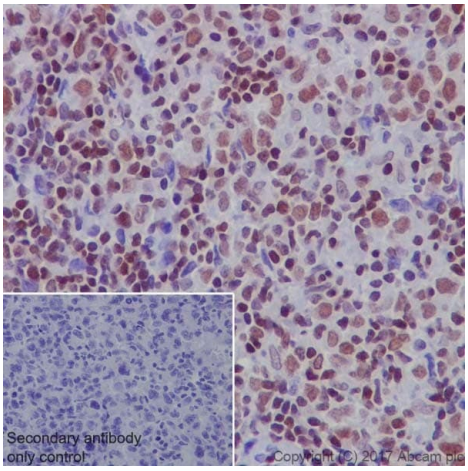
Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (human Burkitt's lymphoma cell line) cells labeling RUNX3 with ab224641 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on Raji cell line. DAPI (blue) and anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution were used as counterstains.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit (**ab150077**) at 1/1000 dilution.

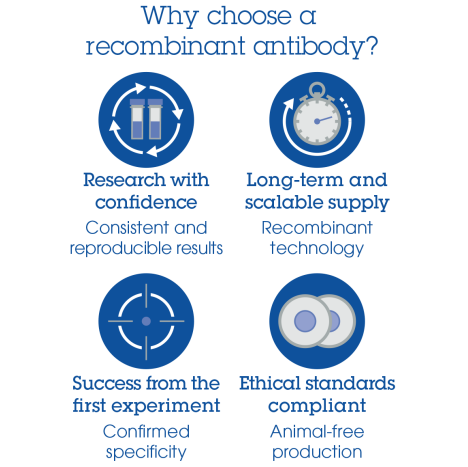


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

Immunohistochemical analysis of paraffin-embedded human diffuse large B cell lymphoma tissue labeling RUNX3 with ab224641 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use. Nuclear staining on human diffuse large B cell lymphoma (PMID:27184221) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), ready to use.

Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

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