abcam

Product datasheet

Anti-RUNX3 antibody [EPR20687] - BSA and Azide free ab227125

KO VALIDATED | Recombinant | RabMAb

10 Images

Overview

Product name	Anti-RUNX3 antibody [EPR20687] - BSA and Azide free
Description	Rabbit monoclonal [EPR20687] to RUNX3 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ChIP, IP, Flow Cyt, IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human RUNX3 aa 150-250. The exact sequence is proprietary. Database link: Q13761
Positive control	IHC: Human stomach tissue.
General notes	Ab227125 is the carrier-free version of ab224641. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. ab227125 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *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 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. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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	EPR20687
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab227125** in the following tested applications.

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

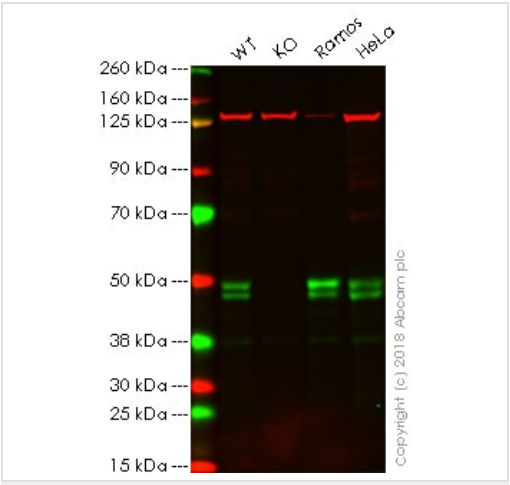
Application	Abreviews	Notes
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 44, 46 kDa (predicted molecular weight: 44 kDa).
ICC/IF		Use at an assay dependent concentration.

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] - BSA and Azide free (ab227125)

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.

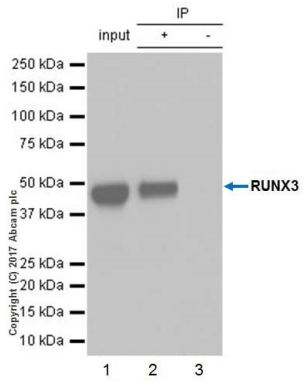
Predicted band size: 44 kDa

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



Immunoprecipitation - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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.

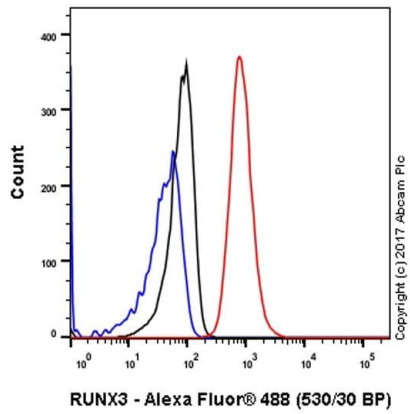
Lane 1: Raji whole cell lysate 10 µg (Input).

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.

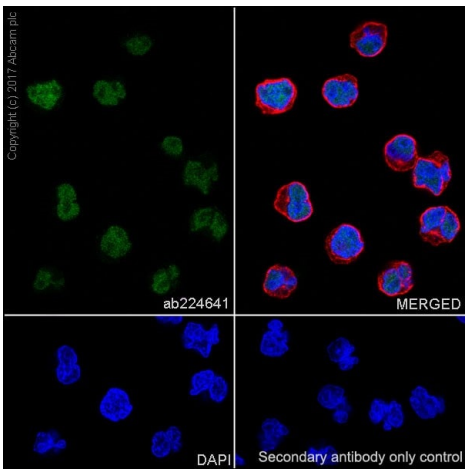
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



Flow Cytometry - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).

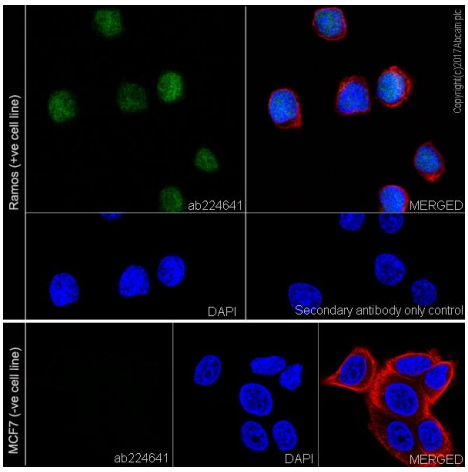


Immunocytochemistry/ Immunofluorescence - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



Immunocytochemistry/ Immunofluorescence - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

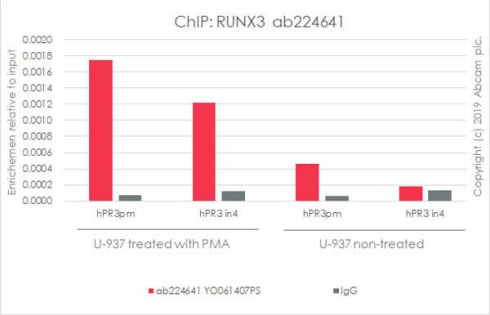
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Ramos (human Burkitt's lymphoma cell line) cells labeling RUNX3 with ab224641 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal 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..

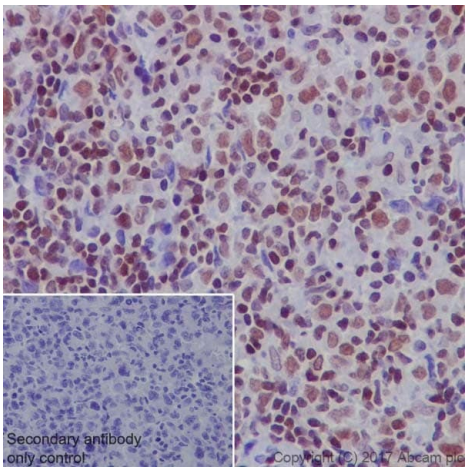
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



ChIP - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



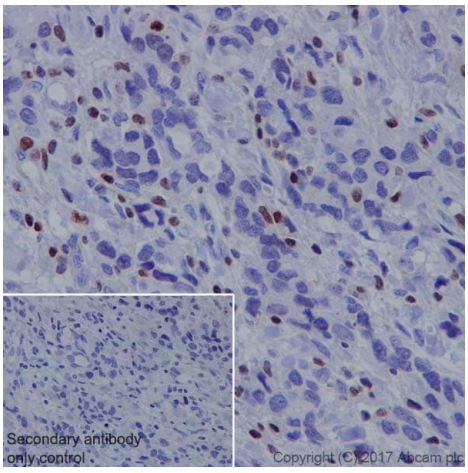
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



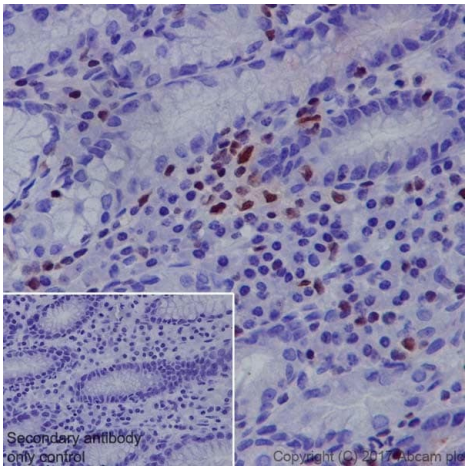
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



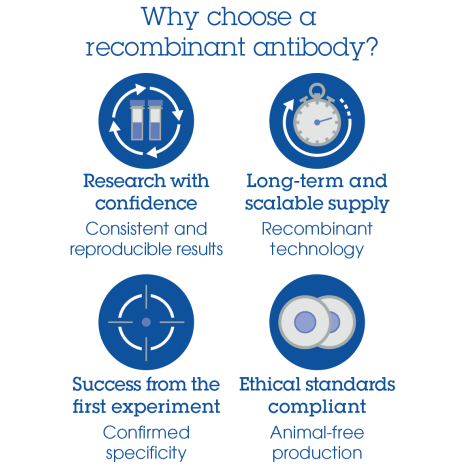
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224641).



Anti-RUNX3 antibody [EPR20687] - BSA and Azide free (ab227125)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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