abcam

ab224641

Anti-RUNX3 antibody [EPR20687] - ChIP Grade

Recombinant | RabMAb | KO Validated | 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	EPR20687
Purification technique	Affinity purification Protein A
Concentration	0.55 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	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	-

Target data

See full target information RUNX3

Function	Forms the heterodimeric complex core-binding factor (CBF) with CBFB. RUNX members modulate the transcription of their target genes through recognizing the core consensus binding sequence 5'-TGTGGT-3', or very rarely, 5'-TGCGGT-3', within their regulatory regions via their runt domain, while CBFB is a non-DNA-binding regulatory subunit that allosterically enhances the sequence-specific DNA-binding capacity of RUNX. The heterodimers bind to the core site of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, LCK, IL3 and GM-CSF promoters (By similarity). May be involved in the control of cellular proliferation and/or differentiation. In association with ZFHX3, up-regulates CDKN1A promoter activity following TGF-beta stimulation (PubMed:20599712). CBF complexes repress ZBTB7B transcription factor during cytotoxic (CD8+) T cell development. They bind to RUNX-binding sequence within the ZBTB7B locus acting as transcriptional silencer and allowing for cytotoxic T cell differentiation. CBF complexes binding to the transcriptional silencer is essential for recruitment of nuclear protein complexes that catalyze epigenetic modifications to establish epigenetic ZBTB7B silencing (By similarity). Necessary for the development and survival of sensory neurons expressing parvalbumin (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	RUNX3 also known as Runt-related transcription factor 3 is a protein that plays an important role in regulating gene expression. The protein has a mass of approximately 44 kDa. RUNX3 is found mainly in tissues like the gastrointestinal tract and neural systems. It functions as a DNA-binding transcription factor that regulates various genes responsible for important cellular processes.
Biological function summary	The function of RUNX3 involves the regulation of cell proliferation differentiation and apoptosis. RUNX3 often forms a complex with the core-binding factor beta (CBFβ) enhancing its stability and DNA-binding capability. This protein is essential in maintaining cellular homeostasis and influencing the immune response particularly through its role in T cell development.
Pathways	RUNX3 is heavily involved in the TGF-beta signaling pathway and the Wnt signaling pathway. It interacts with several proteins such as SMADs in the TGF-beta pathway to modulate gene expression that affects cell growth and survival. Interaction with beta-catenin in the Wnt signaling pathway further emphasizes its importance in regulating cell fate decisions.
Associated diseases and disorders	Alterations in RUNX3 expression or function are linked to cancer including gastric cancer and colorectal cancer. It often shows reduced activity or expression in these cancers which associates with proteins like TGF-beta leading to impaired cell growth control. RUNX3 dysregulation is also connected to neurological disorders due to its role in neural development and function.

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.

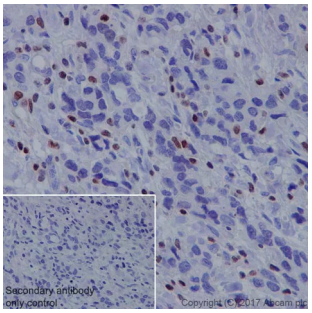
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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11 product images

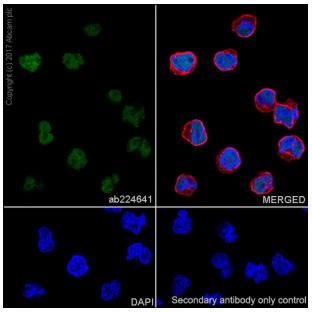


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

RUNX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-RUNX3 antibody

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.

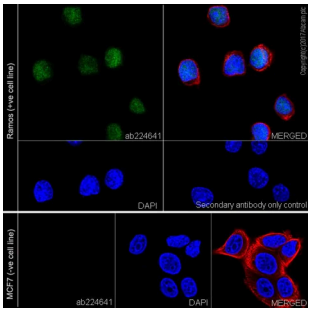
Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



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

RUNX3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RUNX3 antibody

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.



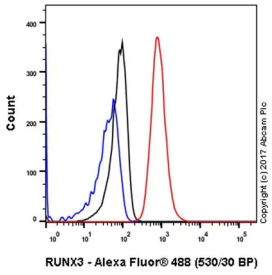
Immunocytochemistry/ Immunofluorescence - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

RUNX3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RUNX3 antibody

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).

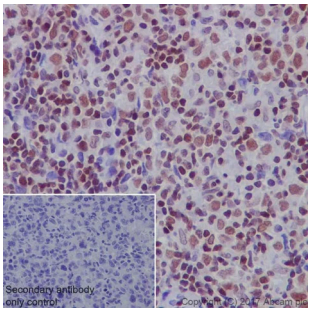
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor® 488 Goat anti-Rabbit (ab150077) at 1/1000 dilution..



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

RUNX3 Flow Cytometry (Intracellular) staining using rabbit Anti-RUNX3 antibody

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.

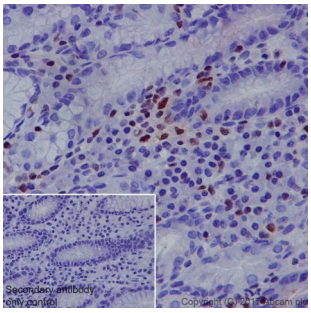


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

RUNX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-RUNX3 antibody

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).

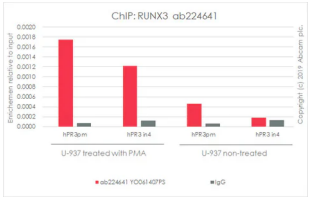


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [EPR20687] - ChIP Grade (ab224641)

RUNX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-RUNX3 antibody

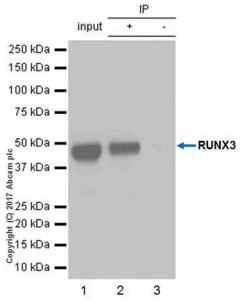
Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling RUNX3 with ab224641 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051), ready to use. Nuclear staining on lymphoid cells of human stomach (PMID:15514019; PMID:21786422) is observed. Counter stained with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



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

Chromatin was prepared from U937 cells treated with 100 nM PMA for 24 hours or left untreated, following the Abcam XChIP 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 (+).

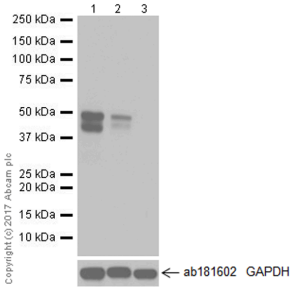
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab224641 in Raji whole cell lysate (-).

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

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

Predicted band size: 44 kDa

Exposure time: 1s



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

RUNX3 Western blot staining using rabbit Anti-RUNX3 antibody

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

Lane 1:
Raji (human Burkitt's lymphoma cell line) cell lysate at 20 µg

Lane 2:
Ramos (human Burkitt's lymphoma cell line) cell lysate at 20 µg

Lane 3:
MCF7 (human breast adenocarcinoma cell line) cell lysate at 20 µg

Secondary

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

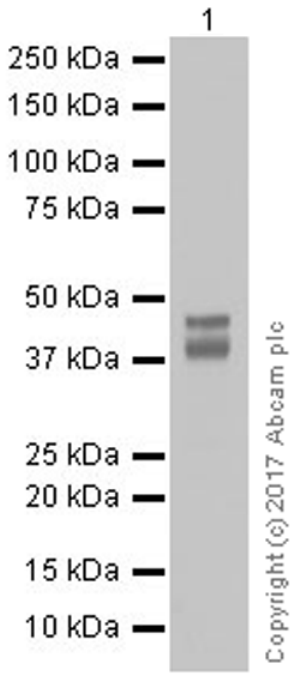
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 44 kDa

Observed band size: 44 kDa, 46 kDa

Exposure time: 5s



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

RUNX3 Western blot staining of Human tonsil tissue lysate using rabbit Anti-RUNX3 antibody

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).

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

All lanes:
Human tonsil tissue lysate at 20 µg

Secondary

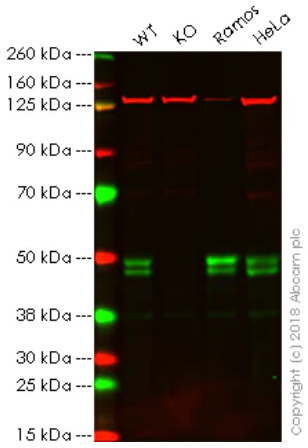
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 44 kDa

Exposure time: 30s



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

RUNX3 Western blot staining using rabbit Anti-RUNX3 antibody

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.

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

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
RUNX3 knockout HAP1 whole cell lysate at 20 µg

Lane 3:
Ramos whole cell lysate at 20 µg

Lane 4:
HeLa whole cell lysate at 20 µg

Predicted band size: 44 kDa

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