abcam

Product datasheet

Anti-RUNX3 antibody [R3-5G4] ab40278

KO VALIDATED

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Overview

Product name	Anti-RUNX3 antibody [R3-5G4]
Description	Mouse monoclonal [R3-5G4] to RUNX3
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human RUNX3 aa 150 to the C-terminus (C terminal).
Positive control	ab40278 gave a positive result in the following whole cell lysates: Jurkat (Human T cell lymphoblast-like cell line) Raji (Human Burkitt's lymphoma cell line) SW480 (Human colon adenocarcinoma cell line)
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. 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, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	R3-5G4

Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40278 in the following tested applications.

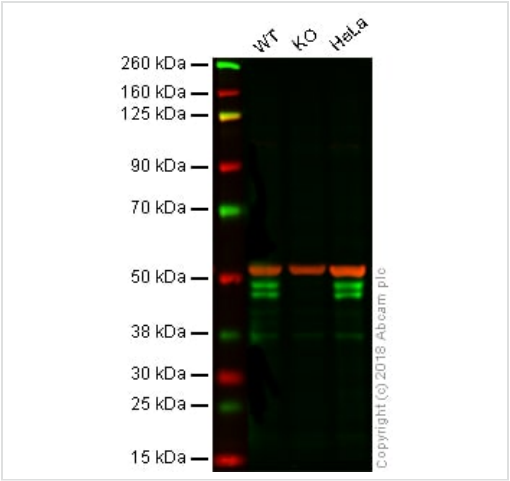
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 44 kDa (predicted molecular weight: 44 kDa).
IHC-P	★☆☆☆☆ (1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval 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

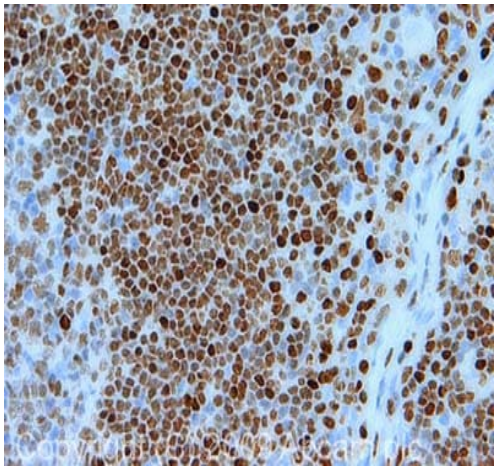


Western blot - Anti-RUNX3 antibody [R3-5G4] (ab40278)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: RUNX3 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)

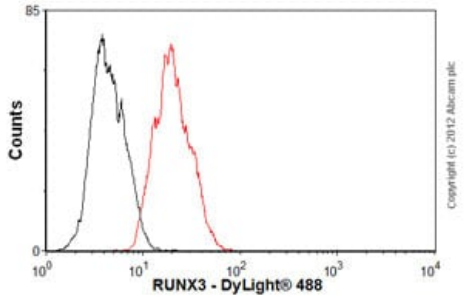
Lanes 1 - 3: Merged signal (red and green). Green - ab40278 observed at 44 kDa. Red - loading control, **ab176560**, observed at 50 kDa.

ab40278 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. ab40278 and **ab176560** (Rabbit anti-alpha Tubulin loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



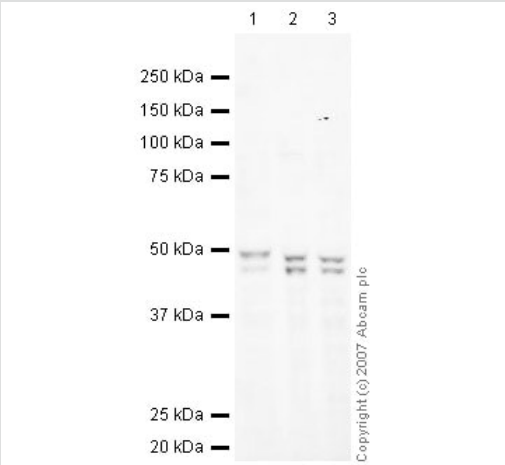
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RUNX3 antibody [R3-5G4] (ab40278)

IHC image of RUNX3 staining in human lymphoma FFPE section, performed on a Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab40278, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Flow Cytometry (Intracellular) - Anti-RUNX3 antibody [R3-5G4] (ab40278)

Overlay histogram showing HEK293 cells stained with ab40278 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40278, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-RUNX3 antibody [R3-5G4] (ab40278)

All lanes : Anti-RUNX3 antibody [R3-5G4] (ab40278) at 1 µg/ml

Lane 1 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 2 : Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate
Lane 3 : SW480 whole cell lysate (**ab3957**)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Rabbit Anti-Mouse IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 44 kDa
Additional bands at: 46 kDa (possible isoform)

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