abcam

Product datasheet

Anti-GATA1 antibody [EPR17362] - ChIP Grade ab181544

KO VALIDATED Recombinant RabMAb

8 References 8 Images

Overview

Product name	Anti-GATA1 antibody [EPR17362] - ChIP Grade
Description	Rabbit monoclonal [EPR17362] to GATA1 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ChIP, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: K562, HEL and MOLT-4 whole cell lysates. IHC-P: Human colon and cervix carcinoma tissues. ICC/IF: K562 cells. IP: K562 whole cell extract. ChIP: Chromatin from K562 cells.
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17362
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab181544 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
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/10000. Detects a band of approximately 43 kDa (predicted molecular weight: 43 kDa).
ChIP		Use 5 µg for 25 µg of chromatin.
IP		1/70.

Target

Function Transcriptional activator which probably serves as a general switch factor for erythroid development. It binds to DNA sites with the consensus sequence [AT]GATA[AG] within regulatory regions of globin genes and of other genes expressed in erythroid cells.

Tissue specificity Erythrocytes.

Involvement in disease Defects in GATA1 are the cause of X-linked dyserythropoietic anemia and thrombocytopenia (XDAT) [MIM:300367]. XDAT is a disorder characterized by erythrocytes with abnormal size and shape, and paucity of platelets in peripheral blood. The bone marrow contains abundant and abnormally small megakaryocytes.
Defects in GATA1 are the cause of X-linked thrombocytopenia with beta-thalassemia (XLTT) [MIM:314050]; also knwon as thrombocytopenia, platelet dysfunction, hemolysis, and imbalanced globin synthesis. XLTT consists of an unusual form of thrombocytopenia with beta-thalassemia. Patients have splenomegaly and petechiae, moderate thrombocytopenia, prolonged bleeding time due to platelet dysfunction, reticulocytosis and unbalanced hemoglobin chain synthesis resembling that of beta-thalassemia minor.
Defects in GATA1 are the cause of anemia without thrombocytopenia X-linked (XLAWT) [MIM:300835]. XLAWT is a form of anemia characterized by abnormal morphology of erythrocytes and granulocytes in peripheral blood, bone marrow dysplasia with hypocellularity of erythroid and granulocytic lineages, and normal or increased number of megakaryocytes. Neutropenia of a variable degree is present in affected individuals.

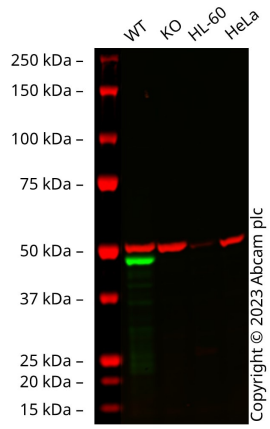
Sequence similarities Contains 2 GATA-type zinc fingers.

Domain The two fingers are functionally distinct and cooperate to achieve specific, stable DNA binding. The first finger is necessary only for full specificity and stability of binding, whereas the second one is required for binding.

Post-translational modifications Highly phosphorylated on serine residues. Phosphorylation on Ser-310 is enhanced on erythroid differentiation. Phosphorylation on Ser-142 promotes sumoylation on Lys-137.
Sumoylation on Lys-137 is enhanced by phosphorylation on Ser-142 and by interaction with PIAS4. Sumoylation by SUMO1 has no effect on transcriptional activity.

Cellular localization Nucleus.

Images



Western blot - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

All lanes : Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544) at 1/10000 dilution

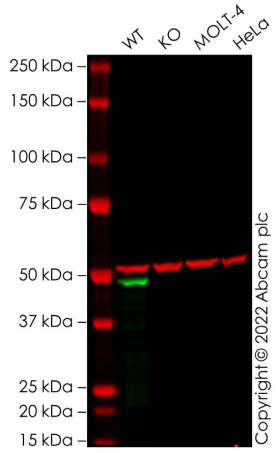
Lane 1 : Wild-type K562 cell lysate
Lane 2 : GATA1 knockout K562 cell lysate
Lane 3 : HL-60 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43 kDa
Observed band size: 47 kDa

False colour image of Western blot: Anti-GATA1 antibody [EPR17362] - ChIP Grade staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab181544 was shown to bind specifically to GATA1. A band was observed at 47 kDa in wild-type K562 cell lysates with no signal observed at this size in GATA1 knockout cell line **ab285360** (knockout cell lysate **ab289686**). To generate this image, wild-type and GATA1 knockout K562 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% 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.



Western blot - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

All lanes : Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544) at 1/10000 dilution

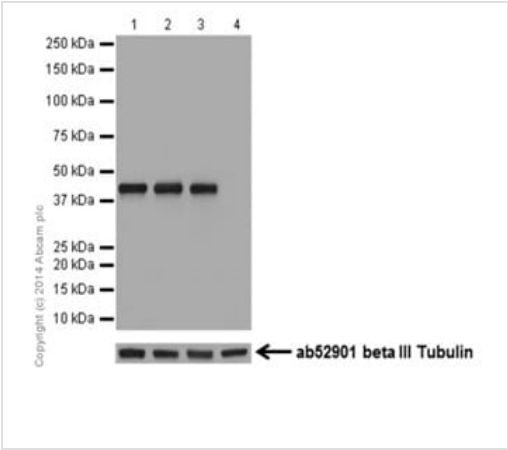
Lane 1 : Wild-type K562 cell lysate
Lane 2 : GATA1 [C116] knockout K562 cell lysate
Lane 3 : MOLT-4 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43 kDa
Observed band size: 48 kDa

False colour image of Western blot: Anti-GATA1 antibody [EPR17362] - ChIP Grade staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab181544 was shown to bind specifically to GATA1. A band was observed at 48 kDa in wild-type K562 cell lysates with no signal observed at this size in GATA1 knockout cell line **ab285360** (knockout cell lysate **ab289686**). To generate this image, wild-type and GATA1 knockout K562 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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.



Western blot - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

All lanes : Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544) at 1/10000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate
Lane 2 : HEL (Human bone marrow erythroleukemia) whole cell lysate
Lane 3 : MOLT-4 (Human lymphoblastic leukemia cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 5 µg per lane.

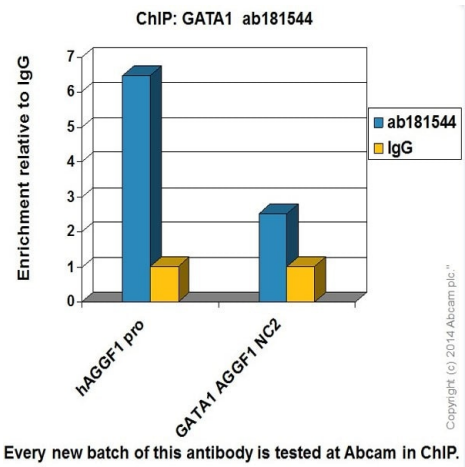
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 15 seconds

HeLa cells do not express GATA1. The expression profile observed in HeLa is consistent with the literature (PMID: 27010793, PMID: 17196618, PMID: 22235304, PMID: 20823267).

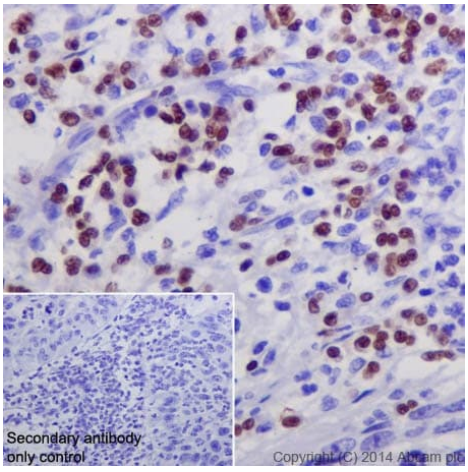
Blocking/Dilution buffer: 5% NFDM/TBST.



ChIP - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

Chromatin was prepared from K562 (Human chronic myelogenous leukemia cells from bone marrow) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab181544 (blue), and 20µl of Anti rabbit IgG sepharose beads. 5µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

"pro" stands for promoter region, while "NC2" stands for negative control which is negative loci at the promoter region.

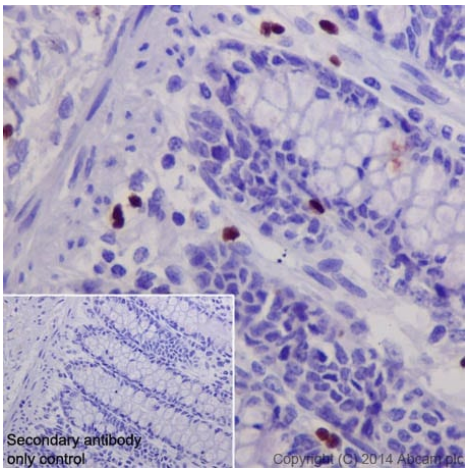


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling GATA1 with ab181544 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on leukocyte of Human cervical cancer is observed. Counterstained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

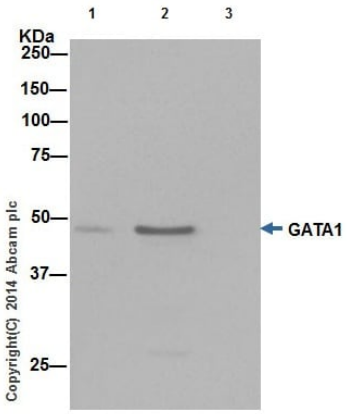


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling GATA1 with ab181544 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear staining on leukocyte of Human colon stroma is observed. Counterstained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

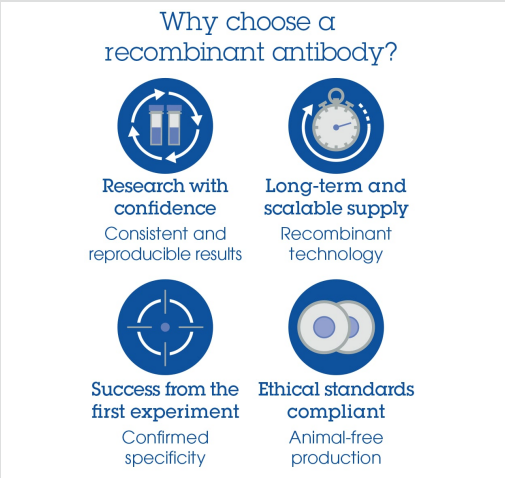
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-GATA1 antibody [EPR17362] - ChIP Grade (ab181544)

GATA1 was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell extract with ab181544 at 1/70 dilution. Western blot was performed from the immunoprecipitate using ab181544 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: K562 whole cell extract 10 µg (Input). Lane 2: ab181544 IP in K562 whole cell extract. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab181544 in K562 whole cell extract. Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Anti-GATA1 antibody [EPR17362] - ChIP Grade

(ab181544)

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