

## Product datasheet

# Anti-GATA3 antibody [EPR16651] - ChIP Grade ab199428

**KO VALIDATED** Recombinant RabMAB

★★★★★ [11 Abreviews](#) [46 References](#) [9 Images](#)

### Overview

<b>Product name</b>	Anti-GATA3 antibody [EPR16651] - ChIP Grade
<b>Description</b>	Rabbit monoclonal [EPR16651] to GATA3 - ChIP Grade
<b>Host species</b>	Rabbit
<b>Specificity</b>	IHC-P is suitable in Human, and may not be suitable for use in Mouse samples.
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), ChIP, WB, IHC-P, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: RAW 264.7 whole cell lysate ( <a href="#">ab7187</a> ), SH-SY5Y cell extracts. EL4, HAP1 and Jurkat whole cell lysates. Mouse brain lysate. IHC-P: Human neuroblastoma and Human breast carcinoma tissue. ICC/IF: SH-SY5Y cells. Flow Cyt (intra): Jurkat cells. ChIP: Chromatin prepared from MCF7 cells.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR16651
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab199428 in the following tested applications.

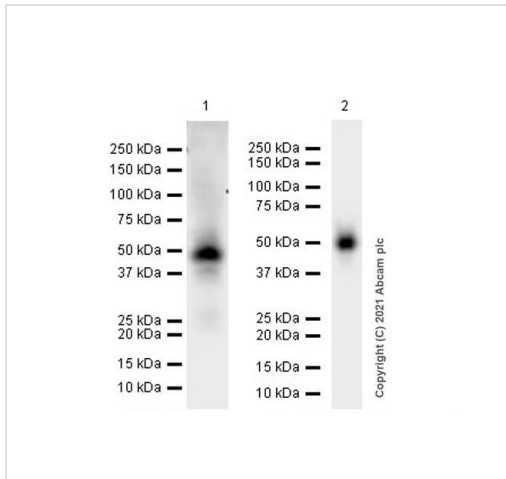
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
ChIP		Use 2 µg for 25 µg of chromatin.
WB		1/1000. Detects a band of approximately 48 kDa (predicted molecular weight: 48 kDa).
IHC-P	★★★★★ (7)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (2)	1/250.

## Target

<b>Function</b>	Transcriptional activator which binds to the enhancer of the T-cell receptor alpha and delta genes. Binds to the consensus sequence 5'-AGATAG-3'.
<b>Tissue specificity</b>	T-cells and endothelial cells.
<b>Involvement in disease</b>	Defects in GATA3 are the cause of hypoparathyroidism with sensorineural deafness and renal dysplasia (HDR) [MIM:146255]; also known as Barakat syndrome.
<b>Sequence similarities</b>	Contains 2 GATA-type zinc fingers.
<b>Cellular localization</b>	Nucleus.
<b>Form</b>	There are 2 isoforms produced by alternative splicing.

## Images



Western blot - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

**All lanes :** Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428) at 1/1000 dilution

**Lane 1 :** Mouse brain lysate

**Lane 2 :** EL4 (mouse lymphoma T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

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

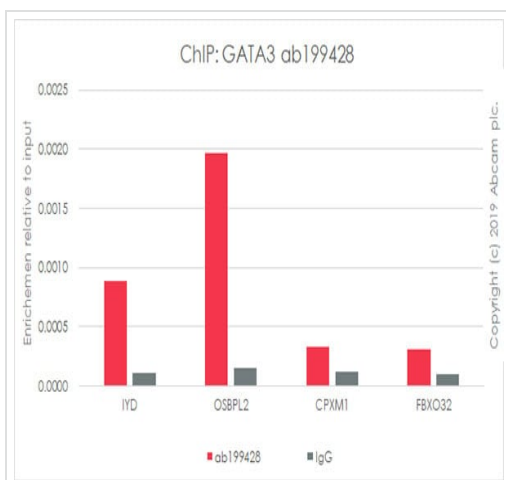
**Predicted band size:** 48 kDa

**Observed band size:** 48 kDa

**Exposure**

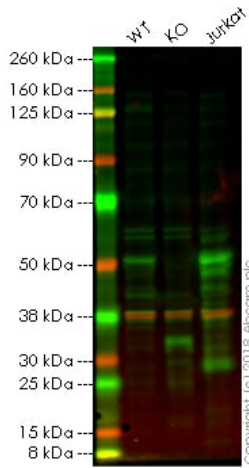
Lane1: 26 seconds

Lane 2: 15 seconds



ChIP - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Chromatin was prepared from MCF7 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab199428 (blue), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the IgG control (yellow). The immunoprecipitated DNA was quantified by real time PCR (SYBR approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

**All lanes :** Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428) at 1/1000 dilution

**Lane 1 :** Wild-type HAP1 whole cell lysate

**Lane 2 :** GATA3 knockout HAP1 whole cell lysate

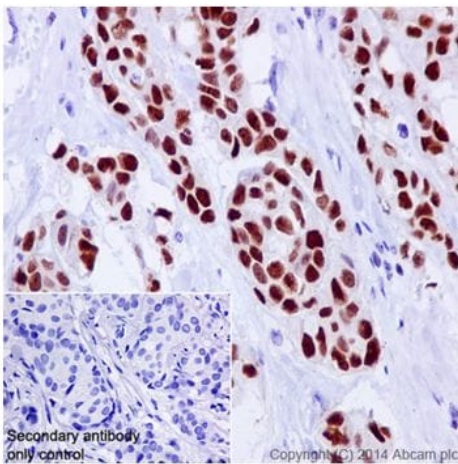
**Lane 3 :** Jurkat whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 48 kDa

**Lanes 1 - 3:** Merged signal (red and green). Green - ab199428 observed at 48 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

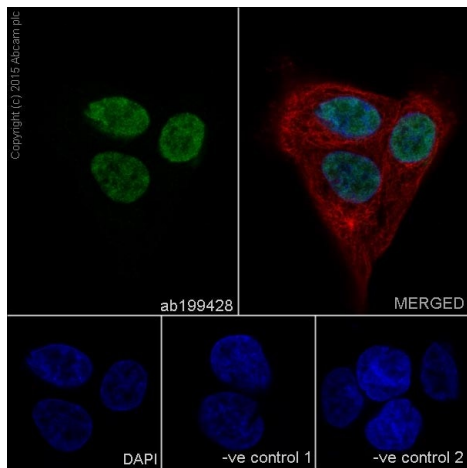
ab199428 was shown to recognize GATA3 in wild-type HAP1 cells as signal was lost at the expected MW in GATA3 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and GATA3 knockout samples were subjected to SDS-PAGE. Ab199428 and **ab9484** (Mouse anti-GAPDH 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling GATA3 with ab199428 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on Human breast carcinoma tissue is observed. Counter stained with Hematoxylin. Negative control: Used PBS instead of primary ab, secondary ab is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**).

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



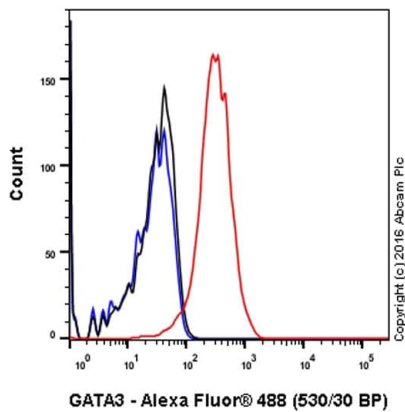
Immunocytochemistry/ Immunofluorescence - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y cells (Human neuroblastoma from bone marrow cells) labeling GATA3 with ab199428 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Nuclear staining on SH-SY5Y cell line is observed. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (Alexa Fluor<sup>®</sup> 594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

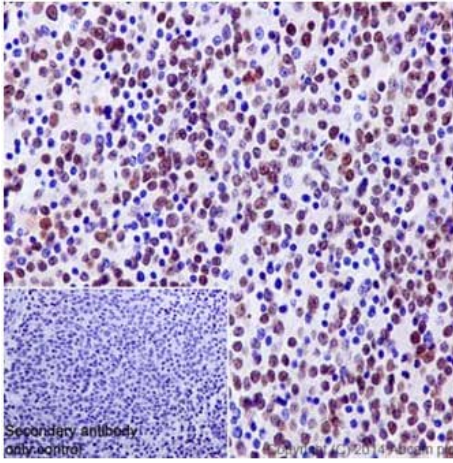
-ve control 1 - ab199428 at 1/250 dilution followed by **ab150120** (Alexa Fluor<sup>®</sup> 594 Goat anti-Mouse secondary) at 1/500 dilution.

-ve control 2 - **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor<sup>®</sup> 488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Flow Cytometry (Intracellular) - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Intracellular Flow Cytometry analysis of Jurkat cells labelling GATA3 with ab199428 at 1/500 (red). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

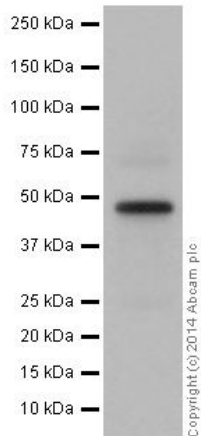


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Immunohistochemical analysis of paraffin-embedded Human neuroblastoma tissue labeling GATA3 with ab199428 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on Human neuroblastoma tissue is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary ab, secondary ab is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**).

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



Western blot - Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428) at 1/1000 dilution + SH-SY5Y (Human neuroblastoma from bone marrow cells) cell extract at 10 µg

**Secondary**

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

**Predicted band size:** 48 kDa

**Observed band size:** 48 kDa

**Exposure time:** 1 minute

Blocking/dilution buffer: 5% NFDM/TBST.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-GATA3 antibody [EPR16651] - ChIP Grade  
(ab199428)

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