abcam

Product datasheet

Anti-GATA3 antibody [EPR16651] - BSA and Azide free ab214804

KO VALIDATED Recombinant RabMAb

1 Abreviews 1 References 11 Images

Overview

Product name	Anti-GATA3 antibody [EPR16651] - BSA and Azide free
Description	Rabbit monoclonal [EPR16651] to GATA3 - BSA and Azide free
Host species	Rabbit
Specificity	IHC-P is suitable in Human, and may not be suitable for use in Mouse samples.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, ChIP, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH-SY5Y, C6 and RAW 264.7 cell extracts. IHC-P: Human neuroblastoma and Human breast carcinoma tissue. ICC/IF: SH-SY5Y cells. Flow Cyt (intra): Jurkat cells. IP: RAW 264.7 whole cell extract.
General notes	ab214804 is the carrier-free version of **ab199428**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. 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. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. 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**.

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	EPR16651
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214804 in the following tested applications.

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

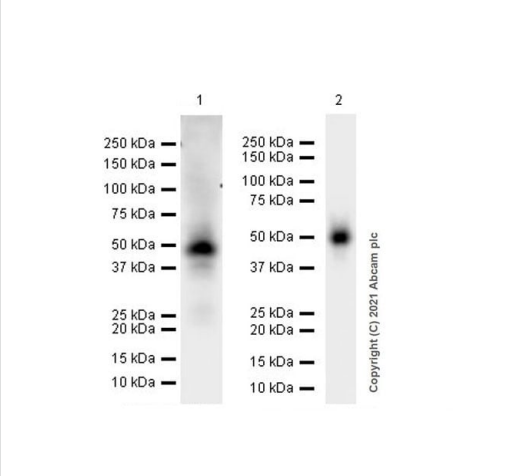
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 48 kDa (predicted molecular weight: 48 kDa).
ChIP		Use at an assay dependent concentration.
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.

Target

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

Form There are 2 isoforms produced by alternative splicing.

Images



Western blot - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

All lanes : Anti-GATA3 antibody [EPR16651] - ChIP Grade (**ab199428**) at 1/100 dilution

Lane 1 : Mouse brain lysate
Lane 2 : EL4 (mouse lymphoma T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

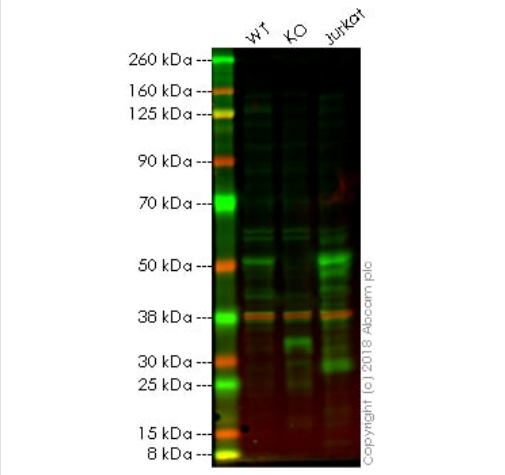
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 48 kDa

Exposure

Lane1: 26 seconds

Lane 2: 15 seconds



Western blot - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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.

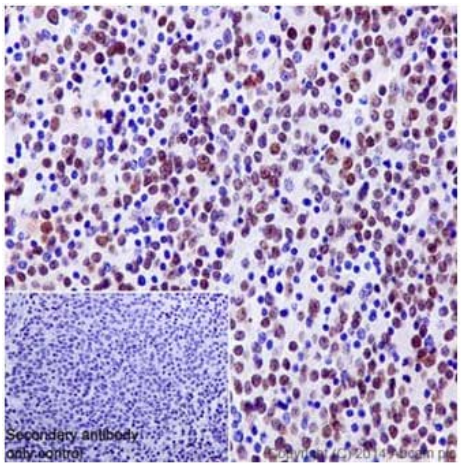
Predicted band size: 48 kDa

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).

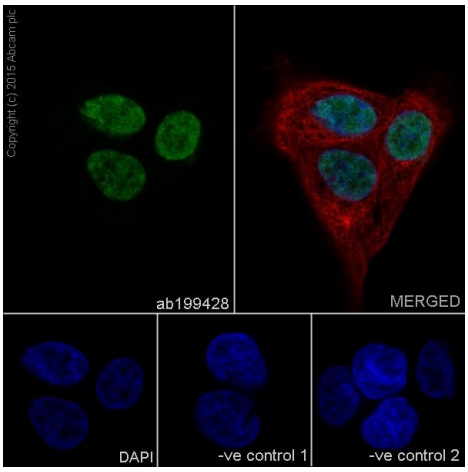


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).

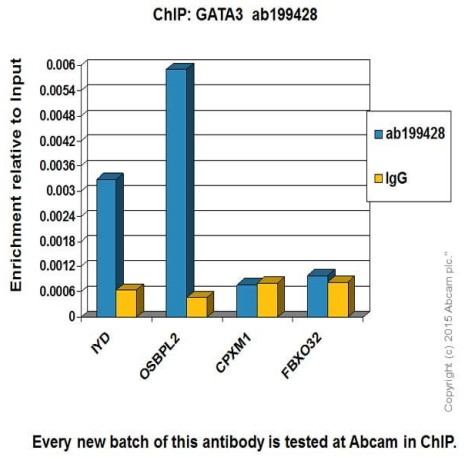
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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® 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® 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® 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® 488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

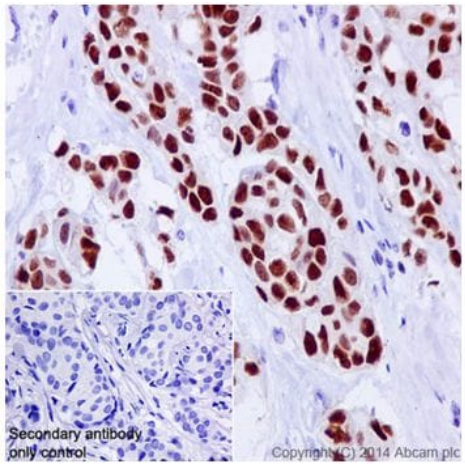
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).



ChIP - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).

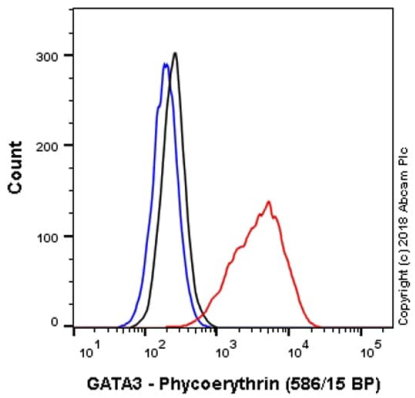


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



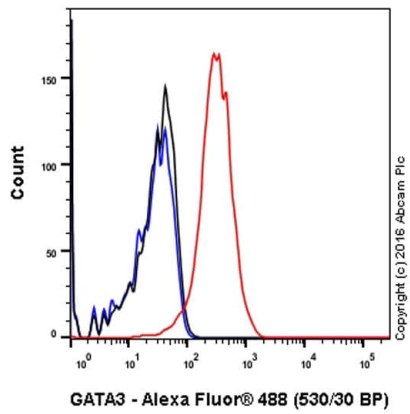
Flow Cytometry (Intracellular) - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

Clone EPR16651 (ab214804) has been successfully conjugated by Abcam. This image was generated using Anti-GATA3 antibody [EPR16651] (PE). Please refer to **ab225419** for protocol details.

Overlay histogram showing MCF7 cells stained with **ab225419** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab225419**, 1/1000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

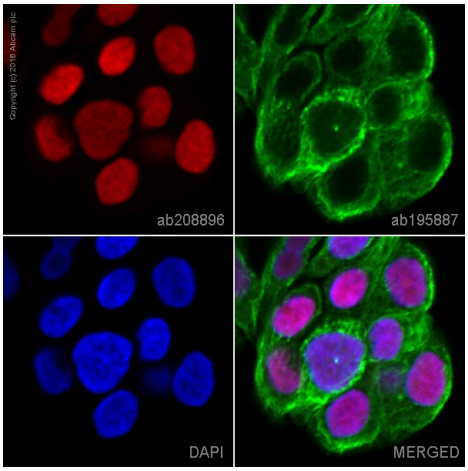
Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



Flow Cytometry (Intracellular) - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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® 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab199428**).



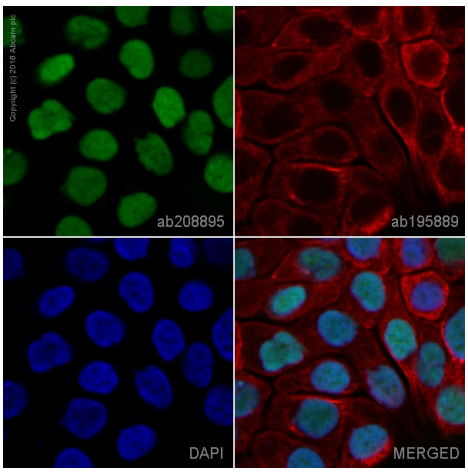
Immunocytochemistry/ Immunofluorescence - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

Clone EPR16651 (ab214804) has been successfully conjugated by Abcam. This image was generated using Anti-GATA3 antibody [EPR16651] (Alexa Fluor® 647). Please refer to **ab208896** for protocol details.

ab208896 staining GATA3 in T47D cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab208896** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in T47D cells fixed with 100% methanol (5 min).



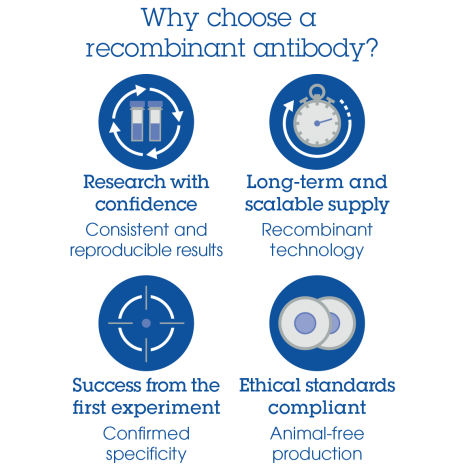
Immunocytochemistry/ Immunofluorescence - Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

Clone EPR16651 (ab214804) has been successfully conjugated by Abcam. This image was generated using Anti-GATA3 antibody [EPR16651] (Alexa Fluor® 488). Please refer to **ab208895** for protocol details.

ab208895 staining GATA3 in T47D cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab208895** at 1/1000 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in T47D cells fixed with 100% methanol (5 min).



Anti-GATA3 antibody [EPR16651] - BSA and Azide free (ab214804)

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