abcam

Product datasheet

Anti-GATA2 + GATA3 antibody [EPR17874] ab182747



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Overview

Product name Anti-GATA2 + GATA3 antibody [EPR17874]

Description Rabbit monoclonal [EPR17874] to GATA2 + GATA3

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human GATA3 full length protein; GATA2 transfected HEK-293 whole cell lysate; HeLa,

> K562, MOLT-4, LNCaP, PC-12 and NIH/3T3 whole cell lysates; Human uterus and fetal kidney lysates. IHC-P: Human prostate cancer, Human neuroblastoma, mouse kidney and rat kidney

tissues. ICC/IF: K562 and HeLa cells. IP: K562 whole cell lysate.

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 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number **EPR17874**

Isotype IgG

Applications

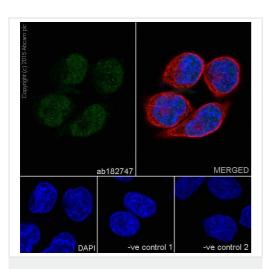
The Abpromise guarantee Our Abpromise guarantee covers the use of ab182747 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 51 kDa (predicted molecular weight: 51,48 kDa).
IHC-P	★★★★☆ (2)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/100.

Target

Images



Immunocytochemistry/ Immunofluorescence - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling GATA2 + GATA3 with ab182747 at 1/1000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).

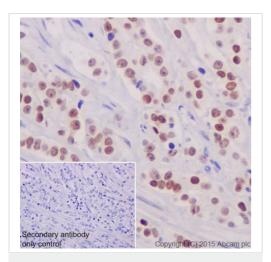
Confocal image showing nuclear and weakly cytoplasmic staining on HeLa cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: ab182747 at 1/1000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

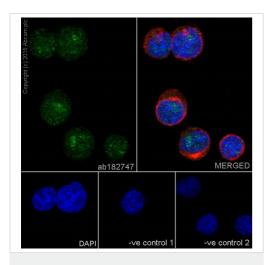
Immunohistochemical analysis of paraffin-embedded Human prostate cancer tissue labeling GATA2 + GATA3 with ab182747 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nucleus staining on cancer cells of Human prostate cancer is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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.



Immunocytochemistry/ Immunofluorescence - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling GATA2 + GATA3 with ab182747 at 1/1000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).

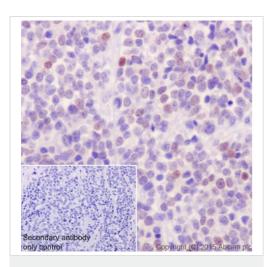
Confocal image showing nuclear and weakly cytoplasmic staining on K562 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: ab182747 at 1/1000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

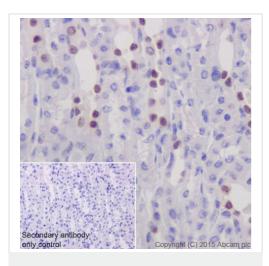
Immunohistochemical analysis of paraffin-embedded Human neuroblastoma tissue labeling GATA2 + GATA3 with ab182747 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Nucleus staining on cancer cells of Human neuroblastoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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 paraffinembedded sections) - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

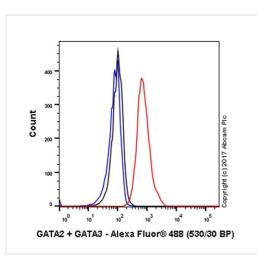
Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling GATA2 + GATA3 with ab182747 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution

Nucleus staining on epithelial cells of mouse renal distal tubule is observed.

Counter stained with Hematoxylin.

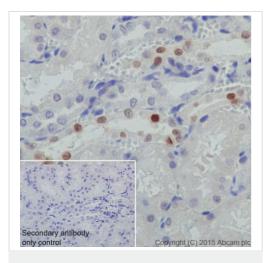
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Flow Cytometry (Intracellular) - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labeling GATA2 + GATA3 with purified ab182747 at 1/150 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

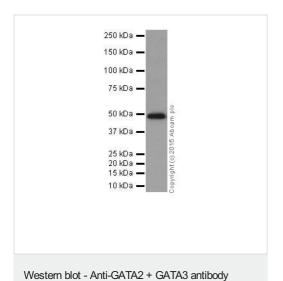
Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling GATA2 + GATA3 with ab182747 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nucleus staining on rat epithelial cells of renal distal tubule is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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.



[EPR17874] (ab182747)

Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/20000 dilution + Human GATA3 full length protein at 0.01 µg

Secondary

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

Predicted band size: 51,48 kDa Observed band size: 48 kDa

Exposure time: 1 minute

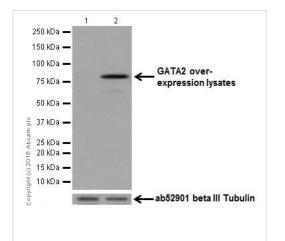
Blocking/Dilution buffer: 5% NFDM/TBST.

Human GATA3 full length protein (epitomics/abcam) contains aa1-443 with N-terminal His-Tag®.

All lanes: Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/50000 dilution

Lane 1: Empty vector (vector control) transfected HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate Lane 2: GATA2 transfected HEK-293 whole cell lysate

Lysates/proteins at 10 µg per lane.



Western blot - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Secondary

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

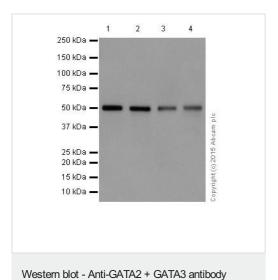
Predicted band size: 51,48 kDa Observed band size: 77 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Human GATA2 over-expression lysates (epitomics/abcam)

contains aa1-480 with GFP-Tag.



[EPR17874] (ab182747)

All lanes: Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/10000 dilution

Lane 1: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lane 2: K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate

Lane 3: MOLT-4 (Human lymphoblastic leukemia cell line) whole cell lysate

Lane 4: LNCaP (Human prostate cancer cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

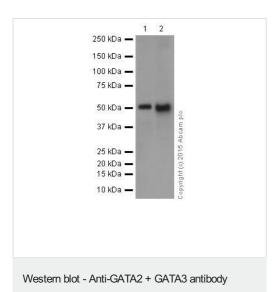
Secondary

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

Predicted band size: 51,48 kDa Observed band size: 51 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



[EPR17874] (ab182747)

All lanes : Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/1000 dilution

Lane 1: Human uterus lysate

Lane 2: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

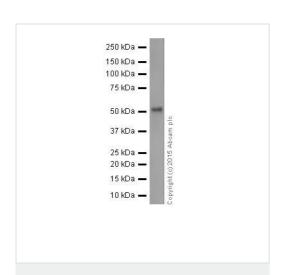
 $\textbf{All lanes:} \ \, \text{Anti-Rabbit lgG (HRP), specific to the non-reduced form}$

of IgG at 1/1000 dilution

Predicted band size: 51,48 kDa **Observed band size:** 51 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/1000 dilution + PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 20 μg

Secondary

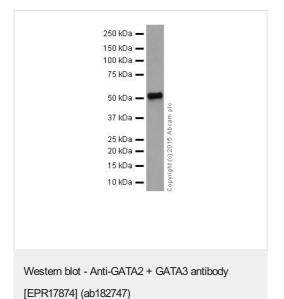
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 51,48 kDa **Observed band size:** 51 kDa

Exposure time: 30 seconds

Western blot - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747) at 1/1000 dilution + NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate at 20 μg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 51,48 kDa **Observed band size:** 51 kDa

Exposure time: 15 seconds

Exposure time. 15 seco

KDa 1 2 3 250— 150—

100— 75—

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■ GATA2+GATA3

Immunoprecipitation - Anti-GATA2 + GATA3 antibody [EPR17874] (ab182747)

Blocking/Dilution buffer: 5% NFDM/TBST.

GATA2 + GATA3 was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate with ab182747 at 1/100 dilution.

Lane 1: K562 whole cell lysate 10ug (Input).

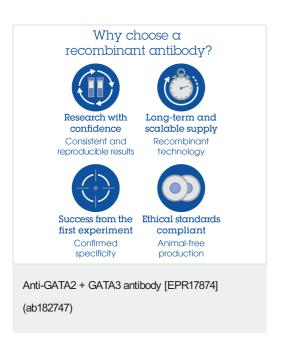
Lane 2: ab182747 IP in K562 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G (\underline{ab172730})$ instead of ab182747 in K562 whole cell lysate.

Western blot was performed from the immunoprecipitate using ab182747 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) was used as secondary antibody at 1/1500.

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

Exposure time: 30 seconds.



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