abcam

Product datasheet

Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free ab244242



★★★★★ 1 Abreviews 13 Images

Overview

Product name Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free

Description Rabbit monoclonal [EPR22102-37] to FOXP3 - BSA and Azide free

Host species Rabbit

Specificity The rat recommendation is based on the IHC results. We do not guarantee WB for rat.

> According to our preliminary WB data, this antibody failed to detect endogenous expression of FOXP3 in the tissues tested in WB, such as thymus. It might work in CD4+CD25+ Treg cells with

abundant FOXP3, but we don't have experimental data to support that.

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

Unsuitable for: ICC/IF or IHC-Fr

Reacts with: Mouse, Rat, Human Species reactivity

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

Positive control WB: HEK-293T transfected with FOXP3 (WT) expression vector with a GFP-myc tag, whole cell

> lysate. Full-length C-MYC/DDK tagged Recombinant Mouse Foxp3 protein. IHC-P: Human, mouse, rat thymus tissues, human and mouse spleen tissues, human tonsil tissue. Flow Cyt (intra):

HEK-293T transfected with a GFP-tagged FOXP3 expression construct. IP: HEK-293T

transfected with a GFP-tagged FOXP3 expression construct whole cell lysate.

General notes ab244242 is the carrier-free version of ab215206.

> 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 cellbased 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 EPR22102-37

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab244242 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
mIHC		Use at an assay dependent concentration.
IHC-P	★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Please adjust the dilution factor according to tissues from different species. Recommend performing IHC staining on a Leica Biosystems BOND® RX instrument assay is
WB		Use at an assay dependent concentration. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa). ab215206 is not suitable for endogenous FOXP3 detection in tissues.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes

Target

Function Probable transcription factor. Plays a critical role in the control of immune response.

Involvement in disease Defects in FOXP3 are the cause of immunodeficiency polyendocrinopathy, enteropathy, X-linked

syndrome (IPEX) [MIM:304790]; also known as X-linked autoimmunity-immunodeficiency syndrome. IPEX is characterized by neonatal onset insulin-dependent diabetes mellitus,

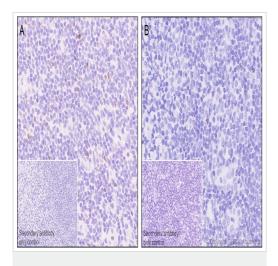
infections, secretory diarrhea, trombocytopenia, anemia and eczema. It is usually lethal in infancy.

Sequence similarities Contains 1 C2H2-type zinc finger.

Contains 1 fork-head DNA-binding domain.

Cellular localization Nucleus.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

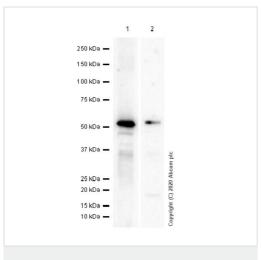
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labelling FOXP3 with **ab215206** at 1:100 dilution (3.67 µg/ml).

Image A: LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody used at a ready to use concentration. Nuclear staining on human tonsil. The section was incubated with <u>ab215206</u> for 30min at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 mins.

Image B: Goat Anti-Rabbit IgG H&L (HRP polymer) secondary antibody used at a ready to use concentration. Nuclear staining on human tonsil. The section was incubated with <u>ab215206</u> overnight at +4 I. Counterstained with Hematoxylin.

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-FOXP3 antibody [EPR22102-37]
- BSA and Azide free (ab244242)

Lane 1: Anti-FOXP3 antibody [EPR22102-37] (**ab215206**) at 1/200 dilution

Lane 2: Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/1000 dilution

All lanes : Full-length C-MYC/DDK tagged Recombinant Mouse Foxp3 protein 10ng

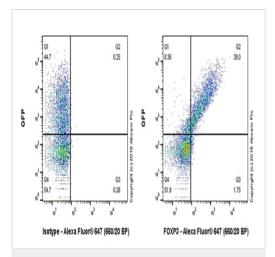
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 47 kDa
Observed band size: 50 kDa

Exposure time: 180 seconds

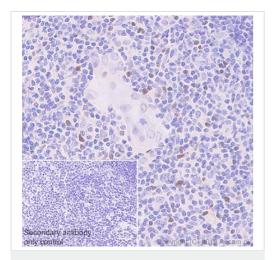
Blocking buffer and concentration: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free (ab244242)

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell line transfected with a GFP-tagged FOXP3 expression construct labeling FOXP3 with ab215206 at 1/40 dilution (Right) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left). Goat Anti-Rabbit IgG H&L (Alexa Fluor [®]647) (ab150079), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215206).

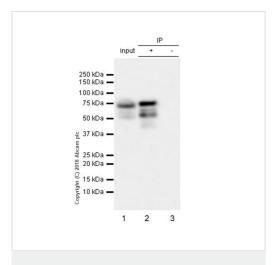


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

Immunohistochemical analysis of paraffin-embedded human thymus tissue labeling FOXP3 with <u>ab215206</u> at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on regulatory T-cells in human thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



Immunoprecipitation - Anti-FOXP3 antibody
[EPR22102-37] - BSA and Azide free (ab244242)

FOXP3 was immunoprecipitated from 0.35 mg of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate with ab215206 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab215206 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate 10 µg (Input).

Lane 2: <u>ab215206</u> IP in HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab215206</u> in HEK-293T cells transfected with a GFP-tagged FOXP3 expression construct whole cell lysate (-).

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

Exposure time: 3 seconds.

FOXP3 isoforms have been described in the literature. (PMID: 19568423).

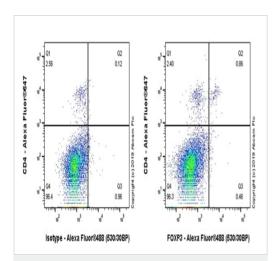
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215206).

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Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free (ab244242)

Human PBMCs were stained with Alexa Fluor[®] 647 conjugated anti-human CD4 and BV421 conjugated anti-human CD25. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear[™] permeabilization buffer, followed by intracellular staining with rabbit IgG (ab172730, Left) or anti-FOXP3 RabMab (Right, 1/40). ab98462, Dylight[®]488-conjugated goat anti-rabbit IgG was used as the secondary at a dilution of 1/2000.

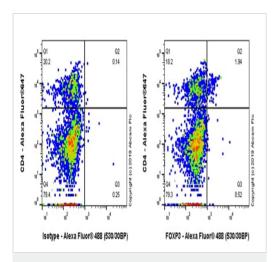
Gating strategy and expression pattern is consistent with literature (PMID: 27330808).



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free (ab244242)

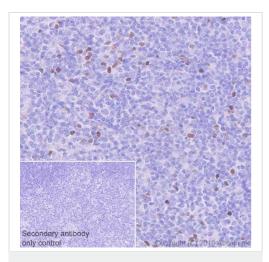
Mouse splenocytes were stained with Alexa Fluor[®]647 conjugated anti-mouse CD4. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear[™] permeabilisation buffer, followed by intracellular staining with rabbit lgG (ab172730, Left) or anti-FOXP3 RabMab (Right, 1/40). Ab150077,Alexa Fluor[®]488-conjugated goat anti-rabbit lgG was used as the secondary at a dilution of 1/2000.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215206).



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] - BSA and Azide free (ab244242)

Rat splenocytes were stained with Alexa Fluor[®]647 conjugated antirat CD4. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear[™] permeabilization buffer, followed by intracellular staining with rabbit lgG (<u>ab172730</u>, Left) or anti-FOXP3 RabMab (Right, 1/40). <u>ab150077</u>, Alexa Fluor[®]488-conjugated goat anti-rabbit lgG was used as the secondary at a dilution of 1/2000.



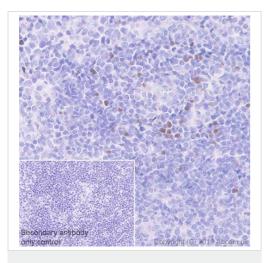
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

Immunohistochemical analysis of paraffin-embedded rat thymus tissue labeling FOXP3 with <u>ab215206</u> at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on regulatory T-cells in rat thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215206).

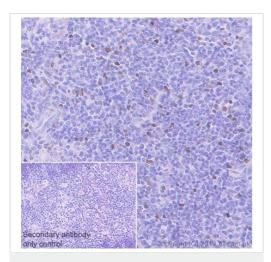


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling FOXP3 with <u>ab215206</u> at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on regulatory T-cells in mouse spleen (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



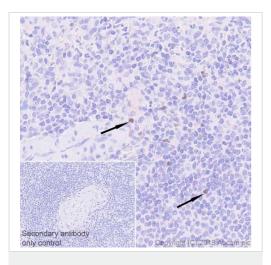
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

Immunohistochemical analysis of paraffin-embedded mouse thymus tissue labeling FOXP3 with ab215206 at 1/1000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in mouse thymus (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215206).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody

[EPR22102-37] - BSA and Azide free (ab244242)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling FOXP3 with ab215206 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on regulatory T-cells in human spleen (PMID: 16380964) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



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