abcam

Product datasheet

Anti-FOXP3 antibody [EPR22102-37] ab215206

יעלאעבע RabMAb

★★★★★ <u>5 Abreviews</u> <u>45 References</u> 画像数 16

製品の概要

製品名 Anti-FOXP3 antibody [EPR22102-37]

製品の詳細 Rabbit monoclonal [EPR22102-37] to FOXP3

由来種 Rabbit

特異性 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.

適用あり: WB, IP, IHC-P, Flow Cyt (Intra), mIHC アプリケーション

適用なし: ICC/IF or IHC-Fr

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール 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.

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル **ウローン名** EPR22102-37

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab215206の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa). 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.
IP		1/30.
IHC-P	★★★★ (5)	1/100 - 1/1000. 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 because BOND® RX instrument assay is stronger than overpight incubation assay.
Flow Cyt (Intra)		1/40.
mIHC		1/100.

追加情報 Is unsuitable for ICC/IF or IHC-Fr.

ターゲット情報

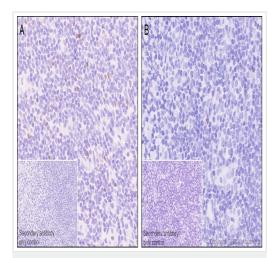
機能
Probable transcription factor. Plays a critical role in the control of immune response.

B連疾患
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.

配列類似性 Contains 1 C2H2-type zinc finger.

Contains 1 fork-head DNA-binding domain.

細胞内局在 Nucleus.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody
[EPR22102-37] (ab215206)

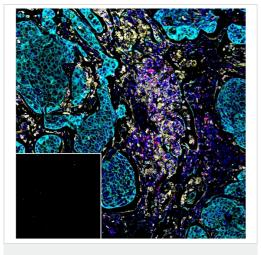
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labelling FOXP3 with ab215206 at 1:100 dilution (3.67 μ g/ml).

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 ab215206 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 ab215206 overnight at +4 **I**. Counterstained with Hematoxylin.

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



Multiplex immunohistochemistry - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

This image is courtesy of TissueGnostics Asia Pacific Limited

10-color fluorescence multiplex immunohistochemical analysis of human lung cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-FOXP3 (ab215206; Cyan; TG540N), anti-PD1 (ab52587; Red; TG700N), anti-CD163 (ab182422; Brown; TG650N), anti-HLA-DR (ab92511; Yellow; TG570N), anti-CD4 (ab133616; Violet; TG620N), anti-CD8 alpha (ab101500; Purple; TG540S), anti-CD20 (ab9475; Grey; TG660S), anti-CD68 (ab192847; Green; TG520N), anti-Cytokeratin 19 (ab52625; Light blue; TG440N). TG470SN (dark blue) was used as a nuclear counter stain. The inset image shows the separate FOXP3 signal.

The section was incubated in nine rounds of staining; in the order of ab215206 (1/100 dilution), <u>ab52587</u> (1/200 dilution), <u>ab182422</u> (1/300 dilution), <u>ab92511</u> (1/200 dilution), <u>ab133616</u> (1/600 dilution), <u>ab101500</u> (1/300 dilution), <u>ab9475</u> (1/100 dilution), <u>ab192847</u> (1/300 dilution), <u>ab52625</u> (1/400 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

Image acquisition was performed with TissueFAXS Spectra (TissueGnostics).

10-color fluorescence multiplex immunohistochemical analysis of human lung cancer tissue (formalin-fixed paraffin-embedded section).

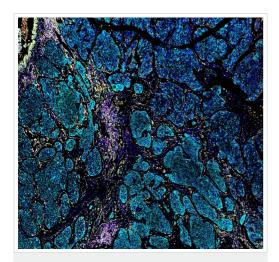
Merged staining of anti-FOXP3 (ab215206; Cyan; TG540N), anti-PD1 (ab52587; Violet; TG700N), anti-CD163 (ab182422; Red; TG650N), anti-HLA-DR (ab92511; Yellow; TG570N), anti-CD4 (ab133616; Orange; TG620N), anti-CD8 alpha (ab101500; Purple; TG540S), anti-CD20 (ab9475; Grey; TG660S), anti-CD68 (ab192847; Green; TG520N), anti-Cytokeratin 19 (ab52625; Light blue; TG440N). TG470SN (dark blue) was used as a nuclear counter stain.

The section was incubated in nine rounds of staining; in the order of ab215206 (1/100 dilution), ab52587 (1/200 dilution), ab182422 (1/300 dilution), ab92511 (1/200 dilution), ab133616 (1/600 dilution), ab101500 (1/300 dilution), ab9475 (1/100 dilution), ab192847 (1/300 dilution), ab52625 (1/400 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

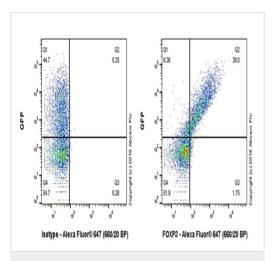
Image acquisition was performed with TissueFAXS Spectra (TissueGnostics).

Intracellular flow cytometric analysis of 4% paraformal dehyde-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.

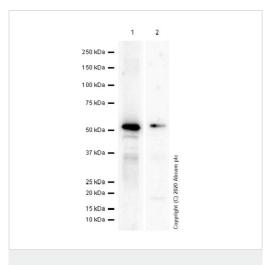


Multiplex immunohistochemistry - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

This image is courtesy of TissueGnostics Asia Pacific Limited



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)



Western blot - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

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 (Anti-DDDDK tag, 1:1000 dilution)

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

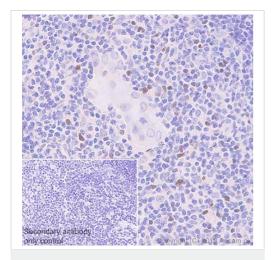
Secondary

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

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

Exposure time: 180 seconds

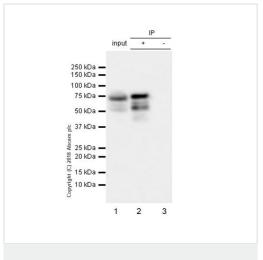
Blocking buffer and concentration: 5% NFDM /TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody
[EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded human thymus tissue labeling FOXP3 with ab215206 at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). 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 (ab209101).

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



Immunoprecipitation - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
10 kDa —
15 kDa —
15 kDa —

Western blot - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

GEP

50 kDa -

37 kDa =

25 kDa -

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: ab215206 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 ab215206 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).

All lanes : Anti-FOXP3 antibody [EPR22102-37] (ab215206) at 1/1000 dilution

Lane 1 : HEK-293T (human embryonic kidney) transfected with an empty vector (vector control), containing a GFP-myc tag, whole cell lysate

Lane 2: HEK-293T transfected with FOXP3 (WT) expression vector containing a GFP-myc tag, whole cell lysate

Lysates/proteins at 10 µg per lane.

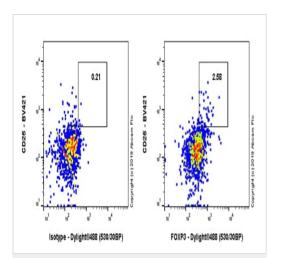
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 47 kDa **Observed band size:** 73 kDa

Exposure time: 6 seconds

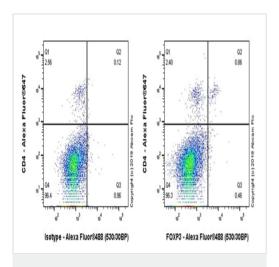
Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

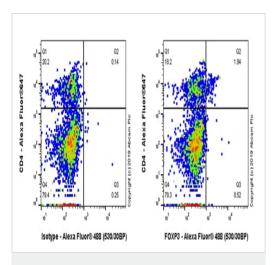
Human PBMCs were stained with Alexa Fluor[®] 647 conjugated anti-human CD4 and BV421 conjugated anti-human CD25. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear[™] permeabilization buffer, followed by intracellular staining with rabbit IgG (ab172730, Left) or anti-FOXP3 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).



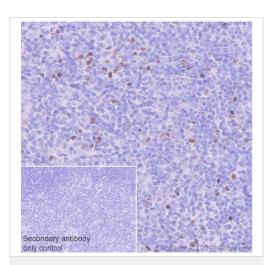
Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

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.



Flow Cytometry (Intracellular) - Anti-FOXP3 antibody [EPR22102-37] (ab215206)

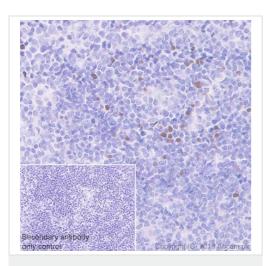
Rat splenocytes were stained with Alexa Fluor[®] 647 conjugated anti-rat CD4. Cells were then fixed with 2% PFA for 10min and permeabilized with True-Nuclear[™] permeabilization buffer, followed by intracellular staining with rabbit lgG (<u>ab172730</u>, Left) or anti-FOXP3 RabMab (Right, 1/40). <u>ab150077</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] (ab215206)

Immunohistochemical analysis of paraffin-embedded rat 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 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 (ab209101).

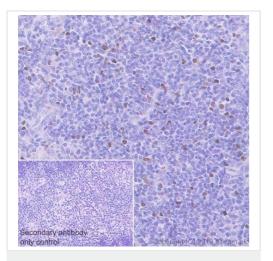
Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody
[EPR22102-37] (ab215206)

Immunohistochemical analysis of paraffin-embedded mouse spleen 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 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).

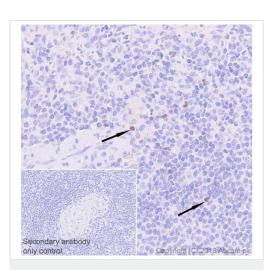
Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody
[EPR22102-37] (ab215206)

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).

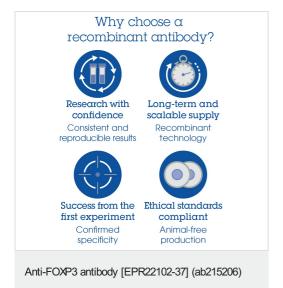
Heat mediated antigen retrieval using <u>ab208572</u> (Universal HIER antigen retrieval reagent).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FOXP3 antibody
[EPR22102-37] (ab215206)

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).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

• Replacement or refund for products not performing as stated on the datasheet

- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.co.jp/abpromise or contact our technical team.

Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors