

Product Datasheet

FoxP3 Antibody - BSA Free

NB600-245

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NB600-245

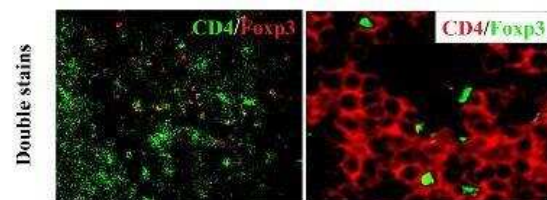
FoxP3 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	47.2 kDa
Product Description	
Host	Rabbit
Gene ID	50943
Gene Symbol	FOXP3
Species	Human, Mouse
Reactivity Notes	This antibody does not appear to detect the mouse FOXP3 protein by western blot, but does work on mouse tissue for IHC.
Immunogen	A synthetic peptide made to the C-terminal region of human FOXP3 (between residues 400-431). [UniProt# Q9BZS1]
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Dual RNAscope ISH-IHC
Recommended Dilutions	Western Blot 1:1000-1:5000, Immunohistochemistry 1:50-1:1000, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 1:200 - 1:1000, Immunohistochemistry-Frozen 1:50, Dual RNAscope ISH-IHC
Application Notes	This FOXP3 antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry on frozen sections and Western Blot. By Western blot, this antibody recognizes bands at ~47.2 kDa representing FOXP3. For IHC-Fr it has been used in mouse thymus from an influenza-infected mouse or thymus from healthy mice (C57BL/6).

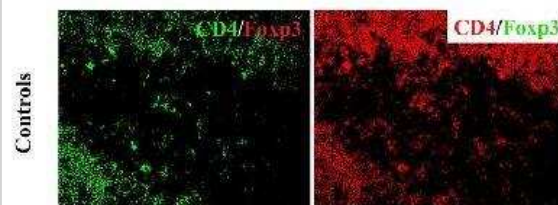


Images

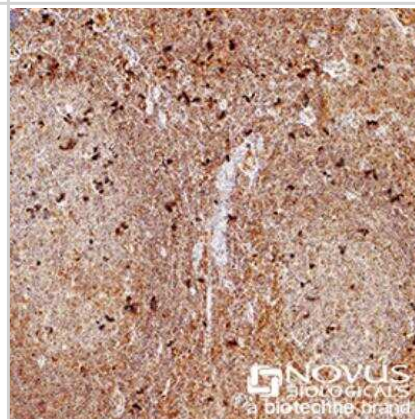
Immunohistochemistry-Frozen: FoxP3 Antibody - BSA Free [NB600-245] - FoxP3 Antibody [NB600-245] - Healthy thymus from a C57BL/6 mouse stained with FoxP3 and CD4.



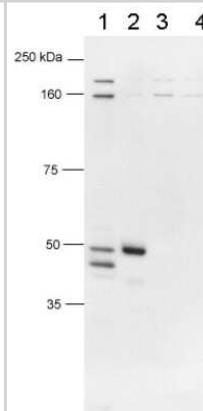
Immunohistochemistry-Frozen: FoxP3 Antibody - BSA Free [NB600-245] - FoxP3 Antibody [NB600-245] - Healthy thymus from a C57BL/6 mouse incubated only with CD4 and no Foxp3.



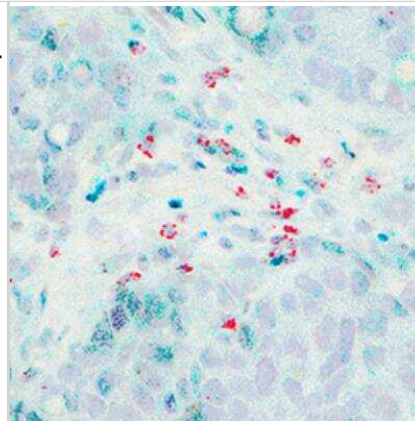
Immunohistochemistry-Paraffin: FoxP3 Antibody [NB600-245] - Fox P3 was detected in immersion fixed paraffin-embedded sections of human tonsil using rabbit anti-human antibody (Catalog # NB600-245) at 1:100 dilution overnight at 4C. Tissue was stained using the VisuCyte anti-rabbit HRP polymer detection reagent (Catalog # VC003) with DAB chromogen (brown) and counterstained with hematoxylin (blue). Images may not be copied, printed or otherwise disseminated without express written permission of Novus Biologicals a bio-techne brand.



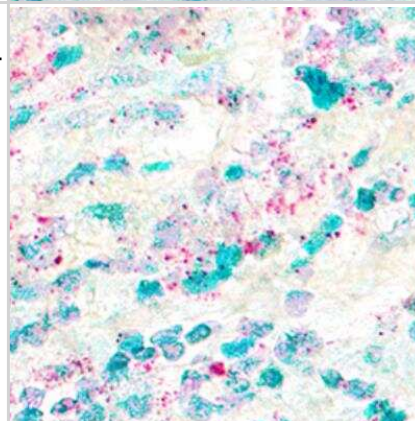
Western Blot: FOXP3 Antibody [NB600-245] - Detection of human FOXP3 using NB 600-245. Lane 1: Human CD4+CD25+ PBL, lane 2: HEK293T transfected with human Foxp3 cDNA, lane 3: 293/mouse foxp3, lane 4: 293/empty vector



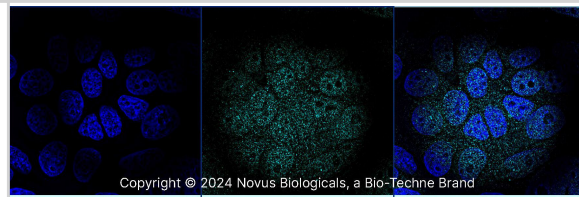
Dual RNAscope ISH-IHC: FoxP3 Antibody [NB600-245] - CD8A mRNA (red) and FOXP3 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human breast cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAscope Probe Hs-CD8A and FOXP3 antibody at 1:100 dilution. Tissue was stained on Leica Bond RX using RNAscope (TM) 2.5 LS Reagent Kit-RED, BOND Polymer Refine Detection (DAB) and Hematoxylin, BOND Polymer Refine Red Detection and Hematoxylin and RNAscope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).



Dual RNAscope ISH-IHC: FoxP3 Antibody [NB600-245] - CD4 mRNA (red) and FOXP3 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human breast cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAscope Probe Hs-CD4 and FOXP3 antibody at 1:200 dilution. Tissue was stained on Leica Bond RX using RNAscope (TM) 2.5 LS Reagent Kit-RED, BOND Polymer Refine Detection (DAB) and Hematoxylin, BOND Polymer Refine Red Detection and Hematoxylin and RNAscope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).



FoxP3 was detected in immersion fixed MCF7 human breast cancer cell line using Rabbit anti-FoxP3 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB600-245AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were stained counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Mitsubishi A, Koyama K, Ogino H et al. Identification of fibrocyte cluster in tumors reveals the role in antitumor immunity by PD-L1 blockade *Cell reports* 2023-02-28 [PMID: 36870329] (ICC/IF, Mouse)

Voss K, Lake C, Luthers CR et al. FOXP3 protects conventional human T cells from premature restimulation-induced cell death *Cell. Mol. Immunol.* 2019-10-28 [PMID: 31659245] (WB, Human)

Schmidleithner, L;Thabet, Y;Schonfeld, E;Kohne, M;Sommer, D;Abdullah, Z;Sadlon, T;Osei-Sarpong, C;Subbaramaiah, K;Copperi, F;Haendler, K;Varga, T;Schanz, O;Bourry, S;Bassler, K;Krebs, W;Peters, AE;Baumgart, AK;Schneeweiss, M;Klee, K;Schmidt, SV;Nussing, S Enzymatic Activity of HPGD in Treg Cells Suppresses Tconv Cells to Maintain Adipose Tissue Homeostasis and Prevent Metabolic Dysfunction *Immunity* 2019-04-15 [PMID: 31027998] (WB, Human)

Katz G, Voss K, Yan TF, Kim YC. FOXP3 renders activated human regulatory T cells resistant to restimulation-induced cell death by suppressing SAP expression. *Cell. Immunol.* 2018-02-12 [PMID: 29454648] (WB, Human)

Rahman S, Gudetta B, Fink J et al. Compartmentalization of immune responses in human tuberculosis: few CD8+ effector T cells but elevated levels of FoxP3+ regulatory t cells in the granulomatous lesions. *Am J Pathol*;174(6):2211-24. 2009-06-01 [PMID: 19435796] (IHC-Fr, Human)

Ochando, JC et al. Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3+ regulatory T cells. *J Immunol*;174(11):6993-7005. 2005-06-01 [PMID: 15905542] (IHC-Fr, Mouse)



Procedures

Immunohistochemistry-Frozen Protocol for FoxP3 Antibody (NB600-245)

FoxP3 Antibody:

Protocol for Foxp3 staining.

- Cut 5 mm sections in a cryostat and fix them in cold acetone for 10 minutes
- Dry the slides completely at room temperature (30 minutes)
- Permeabilize tissues by immersing them in 0.1% saponin, 3 times (5 minutes each time)
- Block unspecific binding to the slides using 5% normal donkey serum, 5% normal rat serum and Fc block (1:500). Everything has to be dissolved in 0.1% saponin
- Incubate slides in a wet chamber at RT for 30 minutes
- Dip off the blocking solution from slides and add streptavidin (0.1 mg/ml) for 15 minutes at RT
- Wash one time with 0.1% saponin
- Add a similar volume of biotin (0.1 mg/ml) and incubate 15 min at RT
- Wash again with 0.1% saponin
- Add rabbit anti-Foxp3 (1:150) and biotin-rat anti-mouse CD4 (1:100) and incubate ON at RT
- Next day: Wash slides with PBS and add SA-594 or SA-488 (dissolved in PBS) to detect CD4 T cells (Dilution 1:500 from molecular probes). Incubate 1 h at RT
- Wash one time with 0.1% saponin and add biotin-donkey anti rabbit (Fab, Jackson Immunoresearch). Secondary antibody is diluted in PBS 1:500 and incubated by 3 hrs.
- Wash with saponin again and add SA-488 or SA-594 (1:500 in PBS). Incubate 1 h at RT
- Wash with PBS 3 times (10 minutes each wash)
- Mount slides with prolong gold antifade (Molecular probes)

Control: Incubated only with CD4 and no Foxp3

Tissue: thymus from an Influenza-infected mouse or thymus from healthy mice (C57BL/6)

Immunohistochemistry-Paraffin Protocol for FoxP3 Antibody (NB600-245)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Western Blot Protocol for FoxP3 Antibody (NB600-245)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.





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Products Related to NB600-245

NBL1-10817	FoxP3 Overexpression Lysate
NB600-245PEP	FoxP3 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

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