

Product datasheet

Anti-FOXP3 antibody [236A/E7] ab20034

★★★★☆ 26 Abreviews 237 References 11 Images

Overview

Product name	Anti-FOXP3 antibody [236A/E7]
Description	Mouse monoclonal [236A/E7] to FOXP3
Host species	Mouse
Specificity	The epitope recognized by this monoclonal antibody is between amino acids 105-236. The antibody is expected to detect full length FOXP3 as well as both cleaved forms.
Tested applications	Suitable for: IHC-P, IHC-Fr, WB, ICC/IF, Flow Cyt
Species reactivity	Reacts with: Human, Cynomolgus monkey, Rhesus monkey
Immunogen	FOXP3 fusion protein.
Positive control	WB: Human thymus and tonsil lysates; HEK-293T overexpressing Human FOXP3 cell lysate. ICC/IF: Head and neck squamous cell carcinoma cells IHC-P: Human tonsil, colon and breast cancer tissue; monkey spleen tissue.
General notes	This FOXP3 antibody was tested in flow cytometry. FOXP3 molecular weight is 50-55 kDa. FOXP3 antibody (ab20034) is expected to detect full length FOXP3 as well as both cleaved forms.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at +4°C. Do Not Freeze.
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein G purified
Clonality	Monoclonal
Clone number	236A/E7
Myeloma	P3-NS1/1-Ag4-1
Isotype	IgG1
Light chain type	kappa

Applications

Our [Abpromise guarantee](#) covers the use of **ab20034** in the following tested applications.

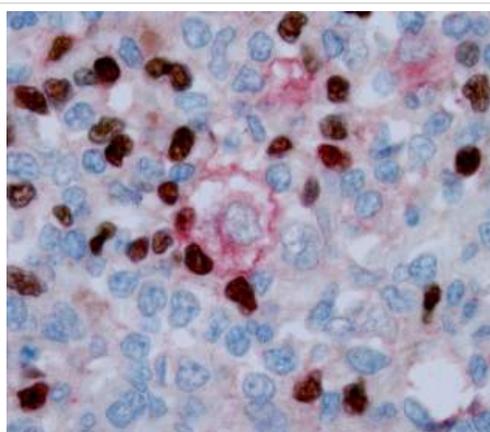
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★★	Use a concentration of 10 µg/ml. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.
IHC-Fr	★★★★☆	Use a concentration of 10 µg/ml.
WB	★★★★☆	Use a concentration of 4 - 5 µg/ml. Detects a band of approximately 50 kDa (predicted molecular weight: 47 kDa).
ICC/IF	★★★★★	Use at an assay dependent concentration.
Flow Cyt		Use 2µg for 10 ⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

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, thrombocytopenia, 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.

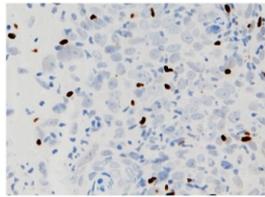
Images



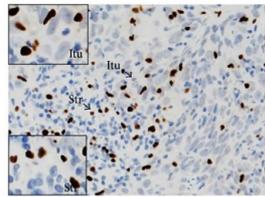
ab20034 used on paraffin embedded human tonsil sections.
Double staining: CD30 in red, FOXP3 in black, blue is haematoxylin counterstaining of nuclei.

As expected FOXP3 stains a sub-population of T cells.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)



(a)

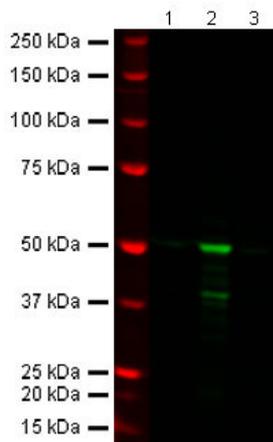


(b)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)

This image is from PubMedId: 27777963. Kaewkangsadan V et al. (2016)

Immunohistochemical analysis of human large and locally advanced breast cancers staining FOXP3 using ab20034. (a) Low level of FOXP3⁺, CTLA-4⁺ Treg infiltration (b) High level of FOXP3⁺ and CTLA-4⁺ Treg infiltration. (ltu: intratumoral Str: stromal)



Western blot - Anti-FOXP3 antibody [236A/E7] (ab20034)

All lanes : Anti-FOXP3 antibody [236A/E7] (ab20034) at 5 µg/ml

Lane 1 : HEK293T cell lysate

Lane 2 : HEK293T cell lysate overexpressing Human FOXP3

Lane 3 : Human tonsil tissue lysate

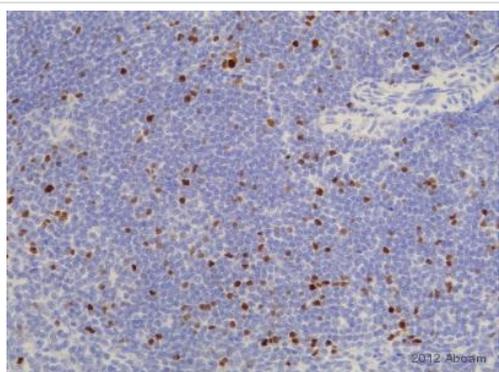
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa

ab20034 detects Human FOXP3 protein at ~50 kDa in HEK293T cells overexpressing the protein. It also detects FOXP3 in Human tonsil tissue lysate, however this band is significantly weaker in endogenous conditions. Upon higher exposure, weak bands can also be observed in HEK293T cell lysate.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with Anti-FOXP3 antibody [236A/E7] (ab20034; 5 microgram per mL) overnight at 4°C. Antibody binding was detected using infrared labelled goat anti-mouse (green; 1:10000) for 1 hour at room temperature before imaging.

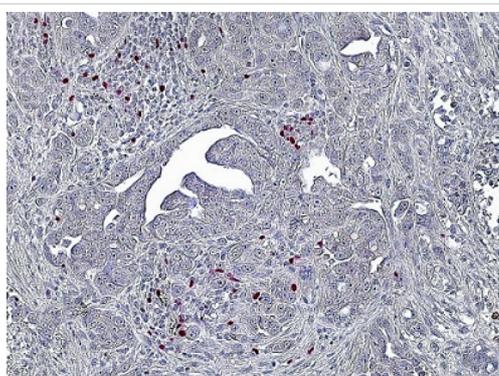


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody

[236A/E7] (ab20034)

Image is courtesy of an AbReview submitted by Jing Ma

ab20034 staining FOXP3 in Cynomolgus Monkey Spleen tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% donkey serum for 20 minutes at room temperature; antigen retrieval was by heat mediation using EDTA, pH9.0. Samples were incubated with primary antibody (1/100) for 30 minutes. A Biotin-conjugated Donkey anti-mouse polyclonal (1/2000) was used as the secondary antibody.



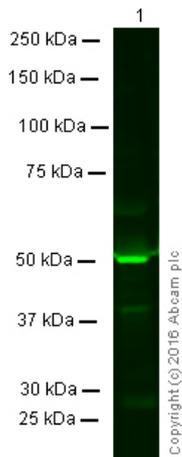
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody

[236A/E7] (ab20034)

Hermans C. et al PLoS One. 2014 Nov 3;9(11):e111757. doi: 10.1371/journal.pone.0111757. eCollection 2014.

FoxP3+ cells mainly accumulate centrally.

The formalin-fixed, paraffin-embedded blocks were cut into approximately <math><2\ \mu\text{m}</math> thick slices and mounted on SuperFrost Plus microscope slides (Menzel Gläser, Braunschweig, Germany). After deparaffinization and rehydration, sections were immersed into Dako Target Retrieval solution (Dako North America Inc., Carpinteria, USA), pH 6, 1/10, incubated at 97°C–99°C at 750 Watt for 2x 15 minutes, and allowed to cool to room temperature for 20 minutes. Endogenous peroxidase activity was blocked by 10-minute incubation in 7.5% hydrogen-peroxide solution (Hydroxgen Peroxide Solution, Sigma Aldrich Co., Munich, Germany). Immunohistochemical staining for FoxP3 (1/180 dilution; for 60 min) was performed according to standard procedure using MACH-3 mouse alkaline phosphatase polymer detection kit from Biocare Medical Systems (Concord, USA). The slides were incubated with monoclonal mouse antibody. Chromogen Red (Dako North America Inc., Carpinteria, USA) was used as chromogen for FoxP3 staining, and lastly hematoxylin counterstaining was done (Vector Laboratories, Burlingame, USA).



Western blot - Anti-FOXP3 antibody [236A/E7] (ab20034)

Anti-FOXP3 antibody [236A/E7] (ab20034) at 5 µg/ml + Human thymus at 20 µg

Secondary

IR-labelled goat anti-mouse (green; 800) at 1/10000 dilution

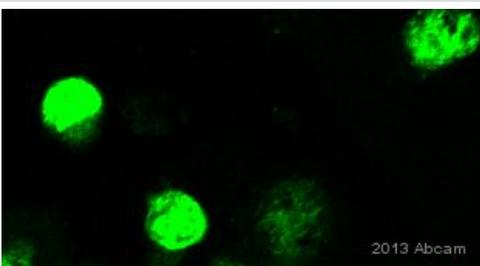
Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 50 kDa

[why is the actual band size different from the predicted?](#)

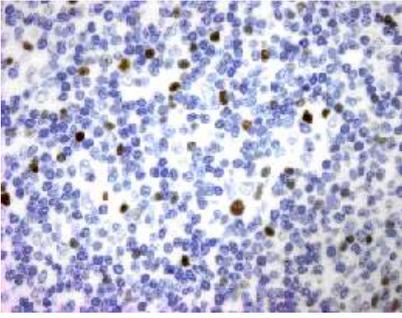
This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with Anti-FOXP3 antibody [236A/E7] (ab20034; 5 microgram per mL), overnight at 4°C. Antibody binding was detected using infrared labelled goat anti-mouse (green; 1:10000) for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FOXP3 antibody [236A/E7] (ab20034)

Image courtesy of an anonymous AbReview

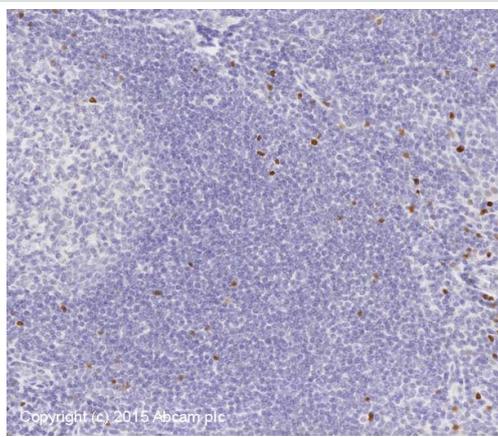
Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized head and neck squamous cell carcinoma cells stained for FOXP3 (green) using ab20034 at 1/100 dilution in ICC/IF.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)

ab20034 used on paraffin embedded human tonsil sections. FOXP3 staining is black, blue is haematoxylin counterstaining of nuclei.

As expected FOXP3 stains a sub-population of T cells.

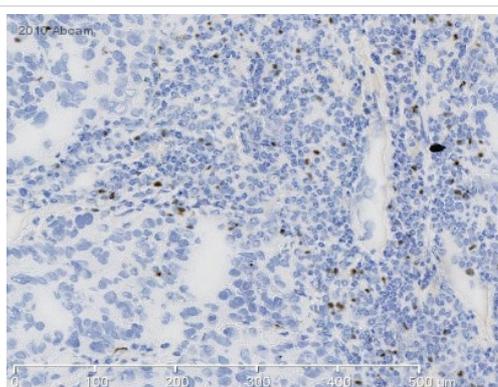


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)

IHC image of Foxp3 staining in Human normal tonsil formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab20034, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

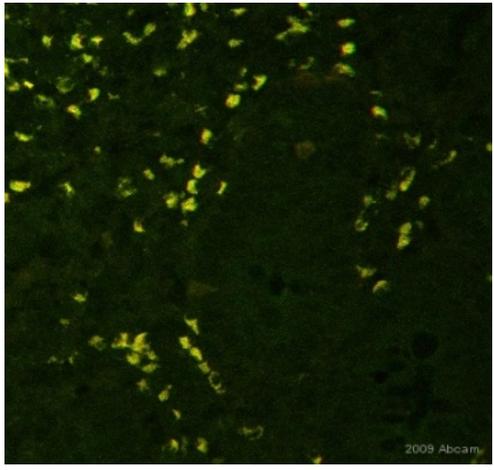
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Frozen sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)
This image is courtesy of an anonymous Abreview

ab20034 staining FOXP3 in Human breast cancer tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with acetone/methanol (1:2), permeabilized with TBS-TritonX-100 and blocked with 10% serum for 10 minutes at 20°C. Samples were incubated with primary antibody (1/50 in PBT (PBS/BSA/Tween)) for 30 minutes at 20°C. An undiluted HRP-conjugated rat anti-mouse/rabbit IgG polyclonal was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)

This image is a courtesy of Nicole Schechter

ab20034 staining FOXP3 in human colon tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent formaldehyde fixation before heat mediated antigen retrieval and then blocking with 10% serum for 2 hours at 21°C. The primary antibody was diluted 1/50 and incubated with sample for 2 hours at 21°C. An Alexa Fluor®488-conjugated rabbit polyclonal to mouse IgG was used undiluted as secondary antibody.

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