

Product datasheet

Anti-FOXP3 antibody [236A/E7] α b20034

Recombinant

★★★★★ [37 Abreviews](#) [537 References](#) [14 Images](#)

Overview

Product name	Anti-FOXP3 antibody [236A/E7]
Description	Mouse monoclonal [236A/E7] to FOXP3
Host species	Mouse
Specificity	The epitope recognized by FOXP3 antibody [236A/E7] (ab20034) is between amino acids 105-236. FOXP3 antibody [236A/E7] (ab20034) is expected to detect full length FOXP3 as well as both cleaved forms.
Tested applications	Suitable for: mIHC, IHC-P, WB
Species reactivity	Reacts with: Human
Immunogen	Recombinant full length protein corresponding to Human FOXP3. Database link: Q9BZS1
Positive control	WB: Human mammary gland lysate, HEK-293 transfected with FOXP3 expression vector containing a GFP-Myc-tag, whole cell lysate. IHC-P: Human tonsil and thymus tissue. mIHC: Human breast cancer tissue.
General notes	This product has switched from a hybridoma to recombinant production method on 20 th January 2021.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	236A/E7
Myeloma	P3-NS1/1-Ag4-1
Isotype	IgG1

Applications

The Abpromise guarantee

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
mlHC		Use at an assay dependent concentration.
IHC-P	★★★★★ (26)	1/500. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 50 kDa (predicted molecular weight: 47 kDa).

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.

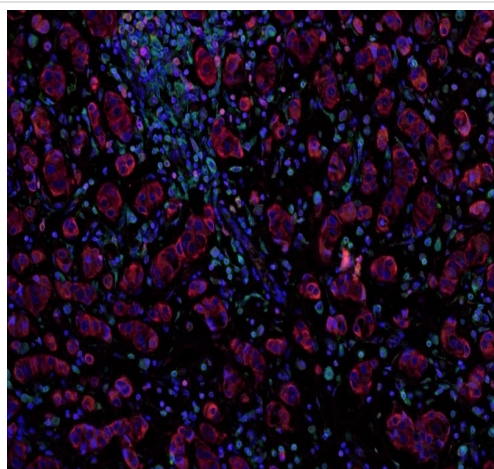
Sequence similarities

Contains 1 C2H2-type zinc finger.
Contains 1 fork-head DNA-binding domain.

Cellular localization

Nucleus.

Images



Multiplex immunohistochemistry - Anti-FOXP3 antibody [236A/E7] (ab20034)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

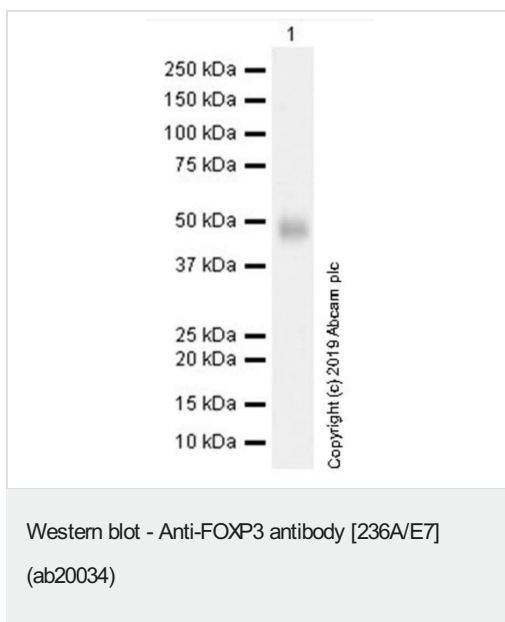
The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for

ab251611 (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.

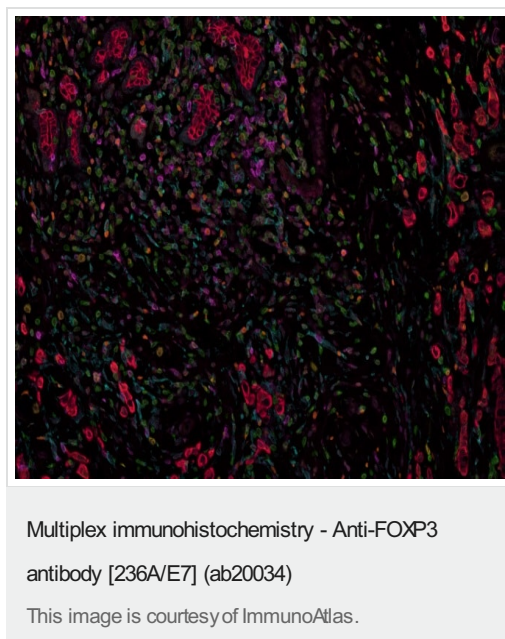
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

This data is courtesy of ImmunoAtlas and it can be found [here](#).



Anti-FOXP3 antibody [236A/E7] (ab20034) at 1/1000 dilution + Human mammary gland lysate at 20 µg

Predicted band size: 47 kDa



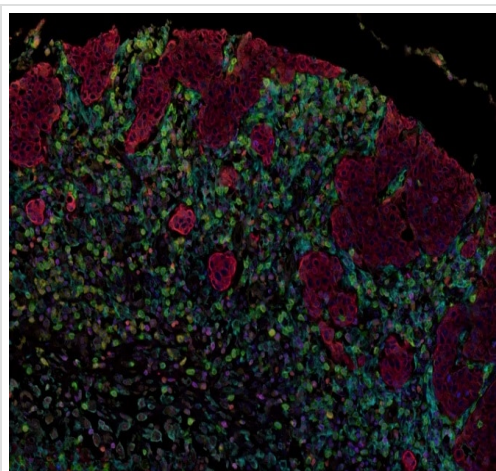
Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

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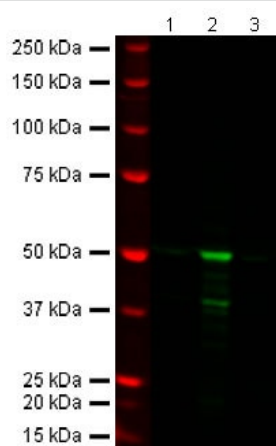
Multiplex immunohistochemistry - Anti-FOXP3 antibody [236A/E7] (ab20034)
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Western blot - Anti-FOXP3 antibody [236A/E7] (ab20034)

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

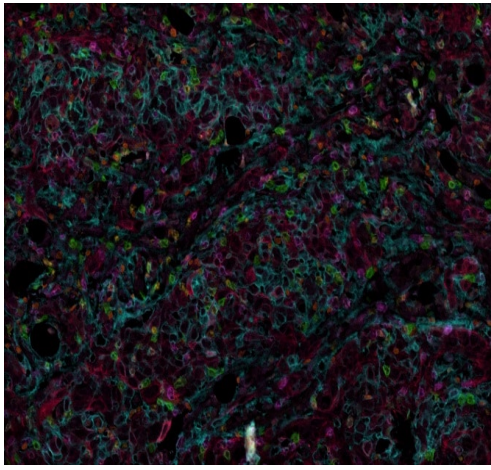
Lysates/proteins at 20 µg per lane.

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.



Multiplex immunohistochemistry - Anti-FOXP3 antibody [236A/E7] (ab20034)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

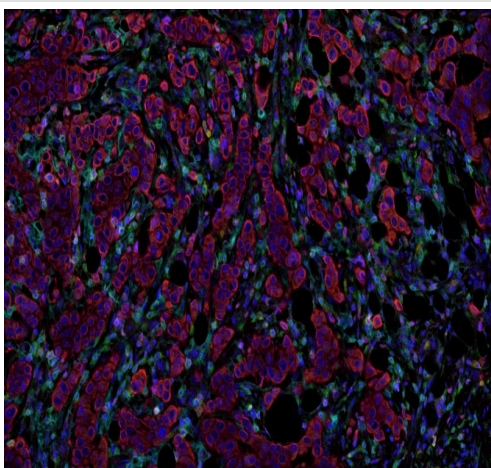
Merged staining of Anti-PD-L1 ([ab251611](#); cyan; Opal™ 520), Anti-Granzyme B ([ab219803](#); yellow; Opal™ 540), Anti-PD1 ([ab251613](#); magenta; Opal™ 570), Anti-pan Cytokeratin ([ab264485](#); red; Opal™ 620), Anti-EpCAM ([ab225894](#); red; Opal™ 620), Anti-CD8 alpha ([ab251596](#); green; Opal™ 650) and Anti-FOXP3 ([ab96048](#); orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

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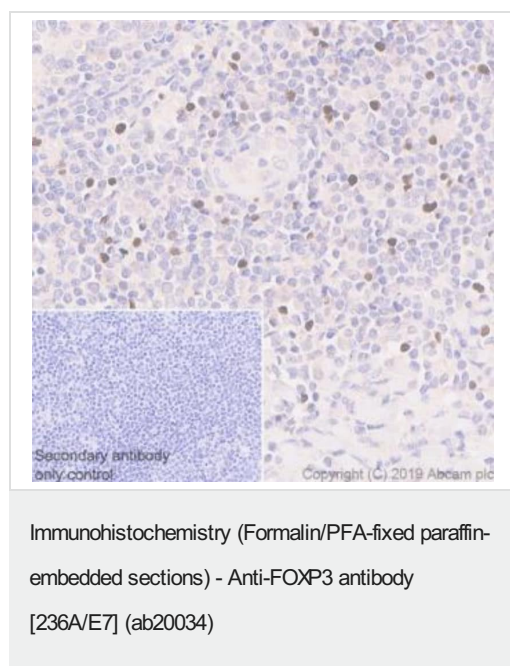
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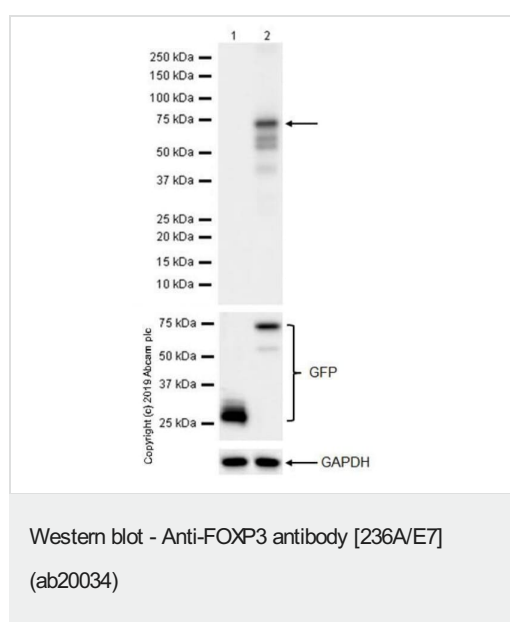
(1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND[®] Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain. Microscopy and pseudocoloring of individual Opal[™] dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences[®]). This data is courtesy of ImmunoAtlas and it can be found [here](#).



Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling FOXP3 with **ab23004** at 1/500 dilution, followed by a ready to use Goat Anti-Mouse IgG. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Mouse IgG.

Perform heat mediated antigen retrieval using Tris/EDTA buffer (pH 9.0).



All lanes : Anti-FOXP3 antibody [236A/E7] (ab20034) at 1/1000 dilution

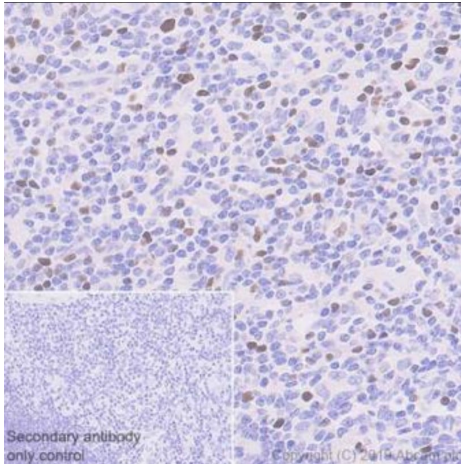
Lane 1 : HEK-293 (human embryonic kidney) transfected with an empty vector (vector control), containing a GFP-Myc-tag, whole cell lysate

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

Lysates/proteins at 10 µg per lane.

Predicted band size: 47 kDa

Exposure time: 1 second

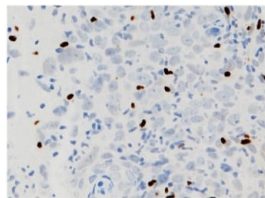


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody [236A/E7] (ab20034)

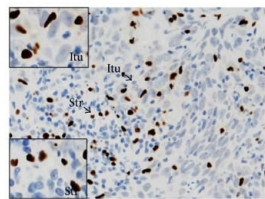
Immunohistochemical analysis of paraffin-embedded human thymus tissue labeling FOXP3 with **ab23004** at 1/500 dilution, followed by a ready to use Goat Anti-Mouse IgG. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Mouse IgG.

Perform heat mediated antigen retrieval using Tris/EDTA buffer (pH 9.0).



(a)



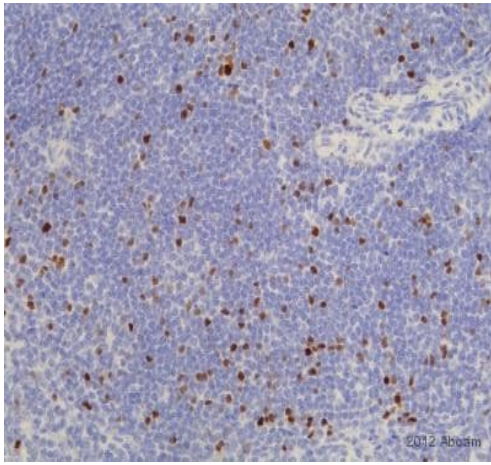
(b)

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

This image is from PubMedId: 27777963.
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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. (Itu: intratumoral Str: stromal)

This image was generated from the hybridoma version of the product.

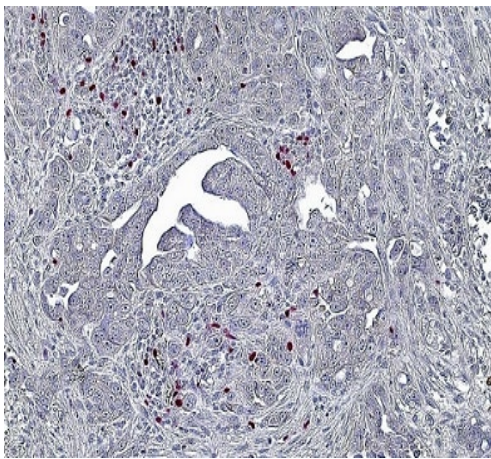


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody
[236A/E7] (ab20034)

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.

This image was generated from the hybridoma version of the product.



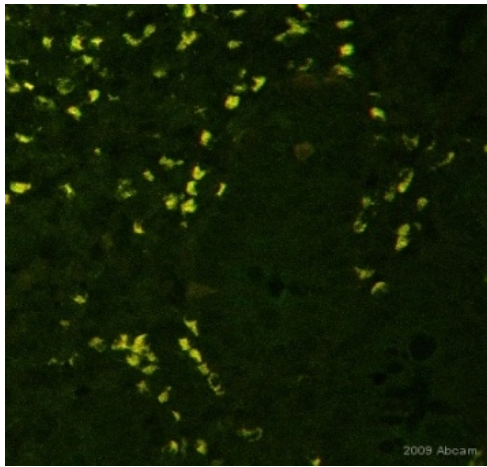
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXP3 antibody
[236A/E7] (ab20034)

Hermans C. et al PLoS One. 2014 Nov 3;9(11):e111757. doi: 10.1371/journal.pone.0111757. eCollection 2014. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

FoxP3+ cells mainly accumulate centrally.

The formalin-fixed, paraffin-embedded blocks were cut into approximately <2 µm 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 (Hydroxene 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).

This image was generated from the hybridoma version of the product.



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.

This image was generated from the hybridoma version of the product.

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