

FOXP3

CONTACT INFORMATION:	Monoclonal Antibodies Unit. Centro Nacional de Investigaciones Oncológicas LRF Haemato-oncology Group. University of Oxford
STATUS:	Validated
TYPE:	mouse anti human
CLONE NAME:	236A
PROTEIN:	Human full length FOXP3
PROTEIN WEB:	http://www.ncbi.nlm.nih.gov/omim/300292
ANTIGEN USED:	GST-FOXP3 recombinant protein
FUSION PARTNER:	myeloma p3-NS1/Ag4-1 (NS1) cells.
ISOTYPE:	IgG1
SPECIES REACTIVITY:	Human
PREPARATION AND STORAGE:	Aliquot and store at 4C. Do not freeze
APP RECOMMENDED:	IHQ-paraffin, IHQ-frozen, IF, WB, Flow cytometry
APP NO TESTED:	IP

DESCRIPTION

FOXP3 is a forkhead-family transcription factor that negatively regulates T cell function. Mice carrying a loss-of-function mutation in FoxP3 present with fatal autoimmune-like disease caused by hyperresponsive CD4(+) T cells. Mice that overexpress scurfin possess fewer mature T cells with reduced functional capabilities compared with normal littermate control mice. FOXP3 is critical for normal CD4+ T cell function and for the successful coordination of a normal response to immunological challenge in vivo.

PUBLICATION DESCRIBING ANTIBODY CHARACTERIZATION/VALIDATION

Roncador G, Brown PJ, Maestre L, Hue S, Martínez-Torrecuadrada JL, Ling KL, Pratap S, Toms C, Fox BC, Cerundolo V, Powrie F, Banham AH.. Analysis of FOXP3 protein expression in human CD4(+)CD25(+) regulatory T cells at the single-cell level. *Eur J Immunol* 2005 35:1681-1691. <http://www.ncbi.nlm.nih.gov/pubmed/15902688>

Banham AH, Lyne L, Scase TJ, Blacklaws BA. Monoclonal antibodies raised to the human FOXP3 protein can be used effectively for detecting Foxp3(+) T cells in other mammalian species. *Vet Immunol Immunopathol.* 2009 Feb 15;127(3-4):376-81.

<http://www.sciencedirect.com/science/article/pii/S0165242708007083>

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Banham AH, Lyne L, Scase TJ, Blacklaws BA. Monoclonal antibodies raised to the human FOXP3 protein can be used effectively for detecting Foxp3(+) T cells in other mammalian species. *Vet Immunol Immunopathol.* 2009 Feb 15;127(3-4):376-81.

Raghavan S, Cao D, Widhe M, Roth K, Herrath J, Engström M, Roncador G, Banham AH, Trollmo C, Catrina AI, Malmström V. FOXP3 expression in blood, synovial fluid and synovial tissue during inflammatory arthritis and intra-articular corticosteroid treatment. *Ann Rheum Dis.* 2008 Dec;68(12):1908-15.

Li, B., Samanta, A., Song, X., Iacono, K.T., Brennan, P., Chatila TA, Roncador G, Banham AH, Riley JL, Wang Q, Shen Y, Saouaf SJ, Greene MI. FOXP3 is a homo-oligomer and a component of a supramolecular regulatory complex disabled in the human XLAAD/IPEX autoimmune disease. *Int Immunol.* 2007 Jul;19(7):825-35.

Ling KL, Pratap SE, Bates GJ, Singh B, Mortensen NJ, George BD, Warren BF, Piris J, Roncador G, Fox SB, Banham AH, Cerundolo V. Increased frequency of regulatory T cells in peripheral blood and tumour infiltrating lymphocytes in colorectal cancer patients. *Cancer Immun.* 2007 Mar 28;7:7.

Enarsson K., Lundgren A., Kindlund B., Hermansson M., Roncador G., Banham A., Lundin S., Quiding-Järbrink, M. Function and recruitment of mucosal regulatory T cells in human chronic *Helicobacter pylori* infection and gastric adenocarcinoma. *Clinical Immunology.* 2006 Dec; 121(3): 358-68.

Li B, Samanta A, Song X, Iacono KT, Brennan P, Chatila TA, Roncador G, Banham AH, Riley JL, Wang Q, Shen Y, Saouaf SJ, Greene MI. FOXP3 is a homo-oligomer and a component of a supramolecular regulatory complex disabled in the human XLAAD/IPEX autoimmune disease

Inter Immun 2007 Jul;19(7):825-35.

Carreras J, Lopez-Guillermo A, Roncador G, Villamor N, Colomo L, Martinez A, Hamoudi R, Howat WJ, Montserrat E, Campo E. High numbers of tumor infiltrating FOXP3-positive regulatory T-cells are associated with improved overall survival in follicular lymphoma. Blood. 2006 Nov 1; 108(9): 2957-64.

Klemke CD, Fritzsching B, Franz B, Kleinmann EV, Oberle N, Poenitz N, Sykora J, Banham AH, Roncador G, Kuhn A, Goerdts S, Krammer PH, Suri-Payer E. Paucity of FOXP3(+) cells in skin and peripheral blood distinguishes Sezary syndrome from other cutaneous T-cell lymphomas. Leukemia. 2006 Jun; 20(6): 1123-9.

Roncador G, Garcia JF, Garcia JF, Maestre L, Lucas E, Menarguez J, Ohshima K, Nakamura S, Banham AH, Piris MA. FOXP3, a selective marker for a subset of Adult T-cell Leukaemia/Lymphoma. Leukemia. 2005 Dec;19(12):2247-53.

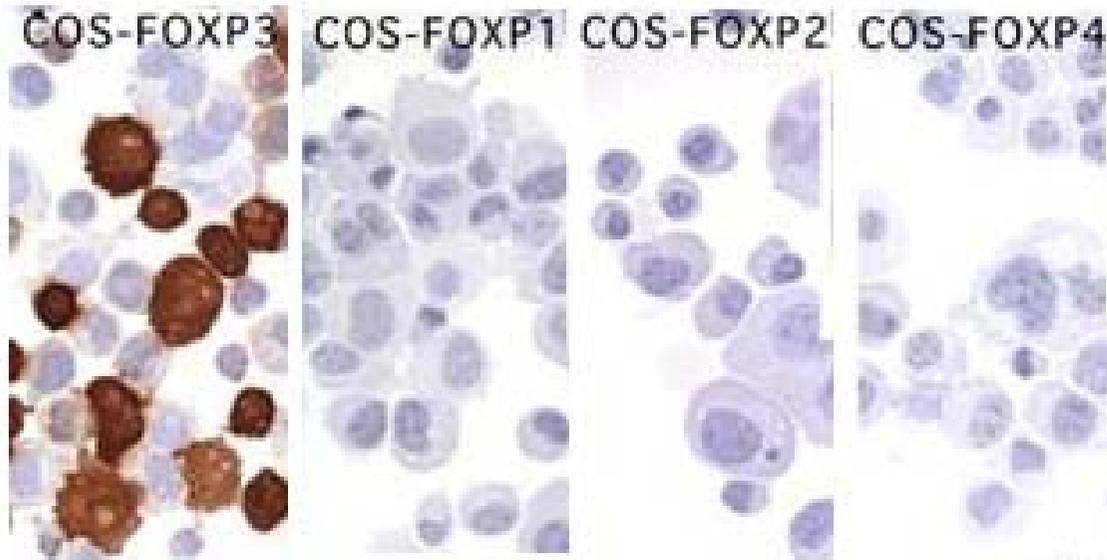
Alvaro T, Lejeune M, Salvadó MT, Bosch R, García JF, Jaén J, Banham AH, Roncador G, Montalbán C, Piris MA. Alvaro T, Lejeune M, Salvadó MT, Bosch R, García JF, Jaén J, Banham AH, Roncador G, Montalbán C, Piris MA. Outcome in Hodgkin s lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. Clin Cancer Res. 2005 11:1467-73.

APPLICATIONS

IHC Techniques	Clone	Dilution	Antibody concentration	Antigen retrieval method	Visualization kit	Positive control	Negative control	Protein localization	Positivity in other species
Frozen tissue and cytopins									
Recommended	236A	supernatant			Goat anti mouse DAKO	Tonsil		Nuclear	Human
Paraffin tissue									

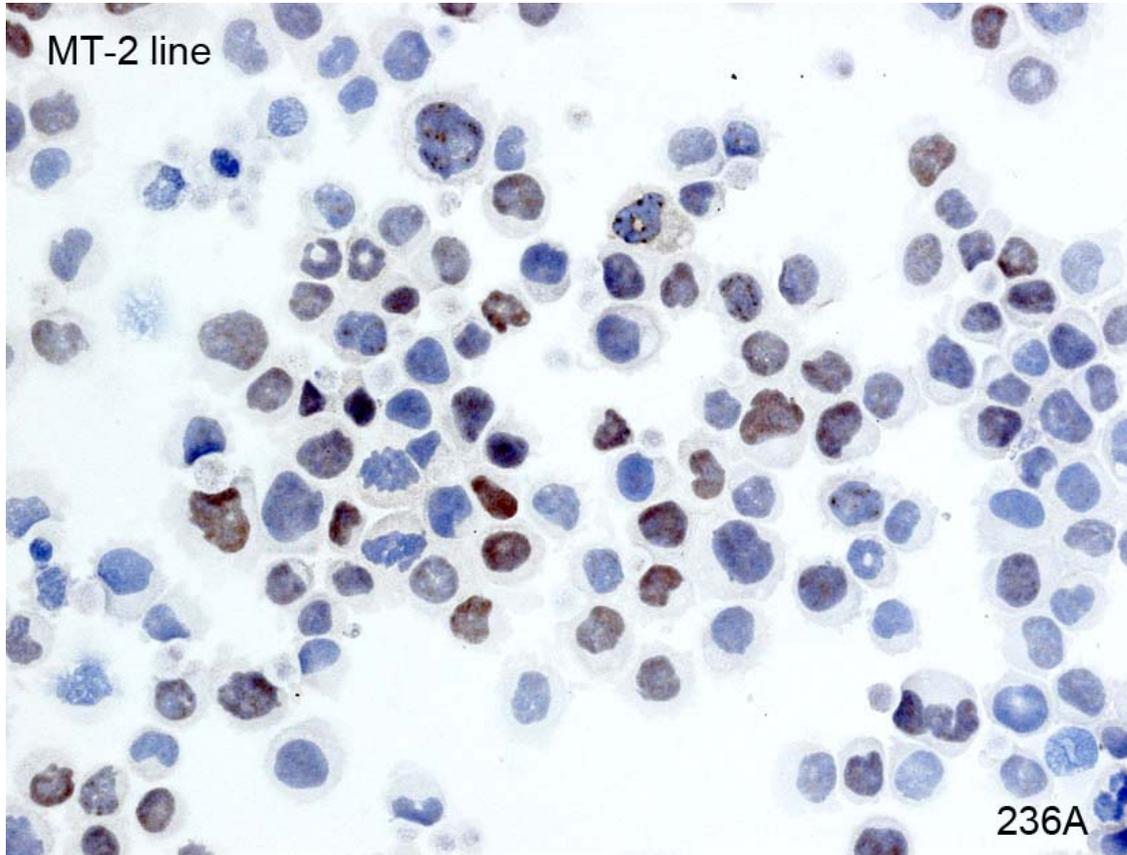
Monoclonal Antibodies Catalogue

Recommended	236A	supernatant		Tris-EDTA	Novolink kit	Tonsil		Nuclear	Human
Immunofluorescence									
Recommended	236A	supernatant		Tris -EDTA	goat anti mouse Alexa	Tonsil		Nuclear	Human



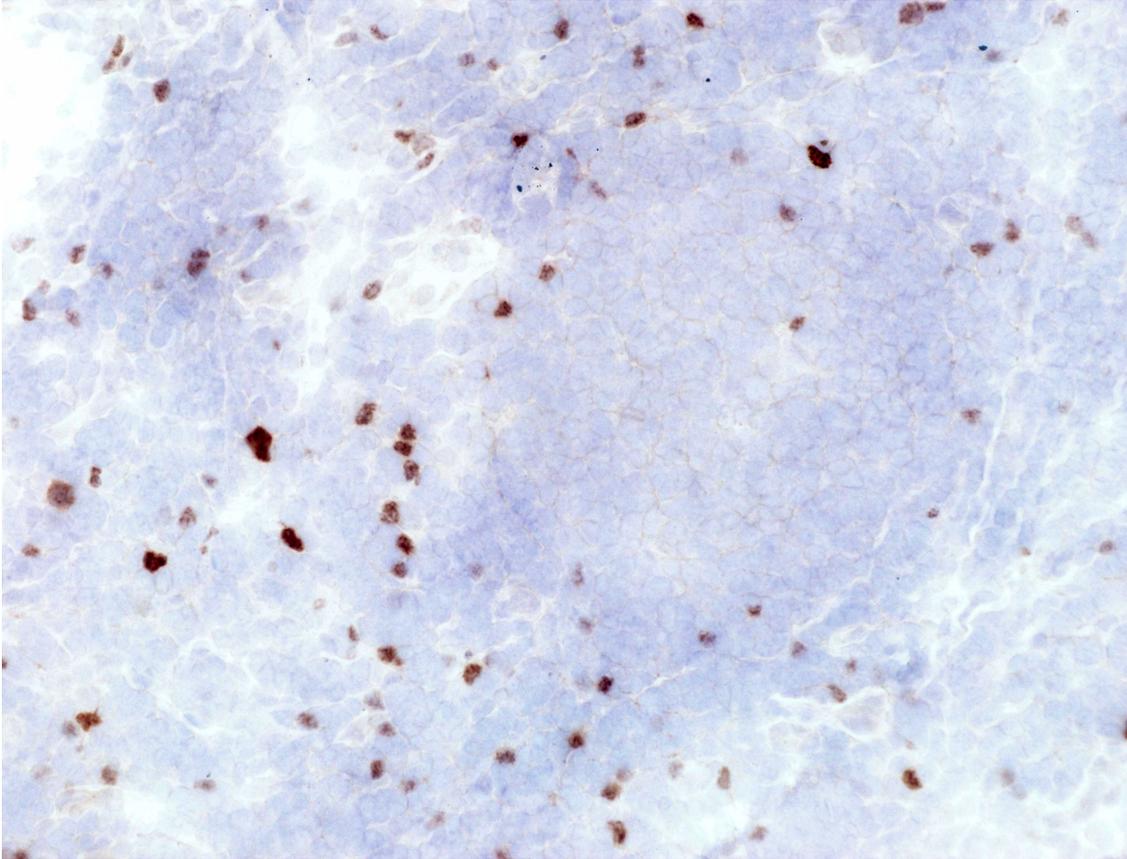
FOXP3 antibody (236A) in transfected cells.

Validation of FOXP3 236A monoclonal antibody in COS-FOXP3, COS-FOXP1, COS-FOXP2 and COS-FOXP4 transfected cells.

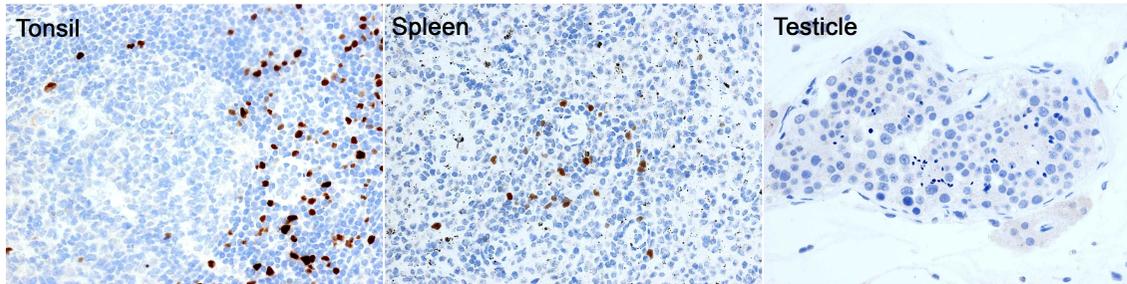


Cytocentrifuge preparation of MT-2 cell line.

MT-2 cell line is a human T cell leukemia virus carrier cell line that express FOXP3 protein.

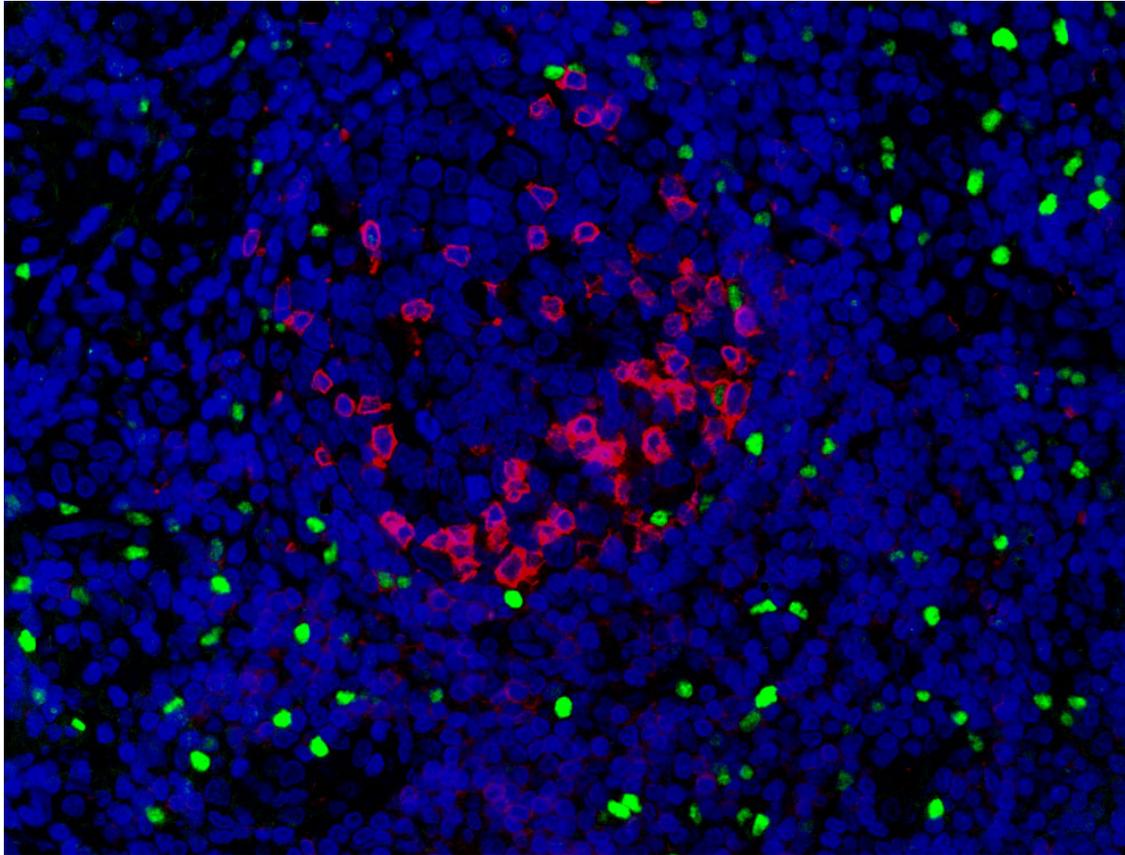


FOXP3 expression in human frozen tonsil.



FOXP3 (236A) expression in human paraffin sections.

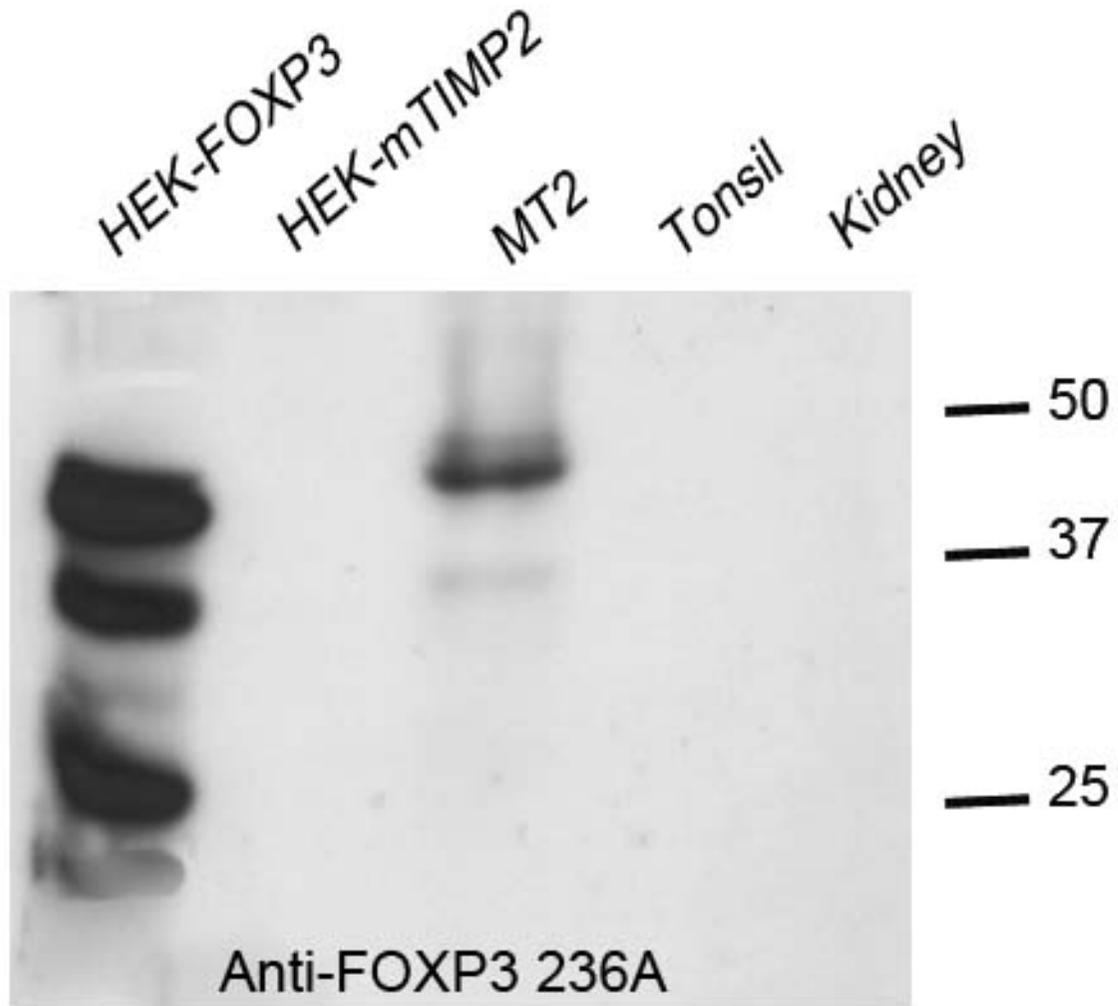
In tonsil, thymus and spleen FOXP3 protein is expressed in the nucleus of regulatory T cell present in the T cell area. Testicle tissue is negative.



FOXP3 (236A) immunofluorescence in human tonsil

Double immunofluorescence staining with FOXP3 (green) and PD-1 (NAT) in red

WB Techniques	Clone	Dilution	Antibody concentration	Positive control	Negative control	Expected MW	Observed Mw	Positivity in other species
Western Blotting								
Recommended	236A	neat	supernatant	MT2 cell line	human kidney	47kDa	47kDa	
Immunoprecipitation								



Western Blotting characterization of 236A monoclonal antibody

Antibody 236A recognises HEK-FOXP3 transfected cells and MT-2 cell line (human T cell leukemia virus carrier cell line) by WB.

Lane 1 HEK-FOXP3 transfected cells (20ug) (+)

Lane 2 HEK-mTIMP2 transfected cells (20ug) (-)

Lane 3 MT2 cell line (150ug) (+)

Lane 4 Human tonsil (150ug) (-)

Lane 5 Human kidney (150ug) (-)

OTHERS	Title	Description
Recommended	Epitope map of anti-FOXP3 (236A) antibody	



Epitope map of anti-FOXP3 (236A) antibody

The figure shows Foxp3 protein functional domains and the location of epitope recognized by 236A antibody (aa105-236). Foxp3 contains a Zn finger/Leucine zipper and a conserved Forkhead Domain.