PD1 (PDCD1)

CONTACT INFORMATION:	Monoclonal Antibodies Unit. Centro Nacional de Investigaciones Oncológicas
STATUS:	Validated
TYPE:	mouse anti human
CLONE NAME:	NAT105
PROTEIN:	Human full length PD1
PROTEIN WEB:	http://www.ncbi.nlm.nih.gov/omim/600244
ANTIGEN USED:	YT cell line
FUSION PARTNER:	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, IP, Flow cytometry

DESCRIPTION

PD1 is a member of the extended CD28/CTLA-4 family of T cell regulators. It contains the immunoreceptor tyrosine-based inhibitory motif (ITIM) and plays a key role in peripheral tolerance and autoimmune disease. It is expressed by germinal center-associated T cells in reactive lymphoid tissue. Immune responses to foreign and self-antigens require specific and balanced responses to clear pathogens and tumors and yet maintain tolerance. Induction and maintenance of T cell tolerance requires PD1, and its ligand PD-L1 on nonhematopoietic cells can limit effector T cell responses and protect tissues from immune-mediated tissue damage. The PD1:PD-L pathway also has been usurped by microorganisms and tumors to attenuate antimicrobial or tumor immunity and facilitate chronic infection and tumor survival.

PUBLICATION DESCRIBING ANTIBODY CHARACTERIZATION/VALIDATION

Roncador, G., Verdes-Montenegro, J.F.G., Tedoldi, S., Paterson, J.C., Klapper, W., Ballabio, E., Maestre, L., Pileri, S., Hansmann, M.L., Piris, M.A., Mason, D.Y., Marafioti, T. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. Haematologica. 2007 Aug;92(8):1059-66. http://www.haematologica.org/content/92/8/1059.full.pdf+html

REFERENCES

Roncador, G., Verdes-Montenegro, J.F.G., Tedoldi, S., Paterson, J.C., Klapper, W., Ballabio, E., Maestre, L., Pileri, S., Hansmann, M.L., Piris, M.A., Mason, D.Y., Marafioti, T. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. Haematologica. 2007 Aug;92(8):1059-66.

Nam-Cha SH, Roncador G, Sanchez-Verde L, Montes-Moreno S, Acevedo A, Domínguez-Franjo P, Piris MA PD-1, a follicular T-cell marker useful for recognizing nodular lymphocyte-predominant Hodgkin lymphoma.
Am J Surg Pathol. 2008 Aug;32(8):1252-7

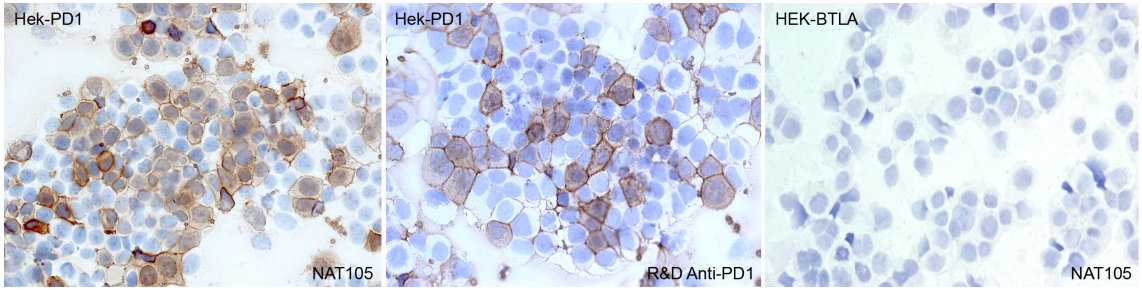
Rodríguez-Pinilla SM, Atienza L, Murillo C, Pérez-Rodríguez A, Montes-Moreno S, Roncador G, Pérez-Seoane C, Domínguez P, Camacho FI, Piris MA. Peripheral T-cell Lymphoma With Follicular T-cell Markers. Am J Surg Pathol. 2008. Dec;32(12):1787-99.

Rodríguez Pinilla SM, Roncador G, Rodríguez-Peralto JL, Mollejo M, García JF, Montes-Moreno S, Camacho FI, Ortiz P, Limeres-González MA, Torres A, Campo E, Navarro-Conde P, Piris MA. Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma expresses follicular T-cell markers. Am J Surg Pathol. 2009 Jan;33(1):81-90.

APPLICATIONS

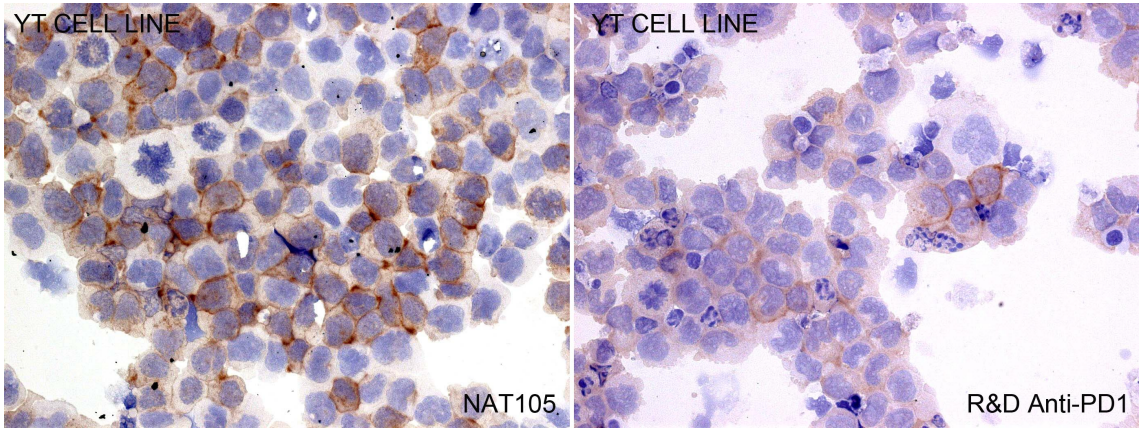
IHC Techniques	Clone	Dilution	Antibody concentration	Antigen retrieval method	Visualization kit	Positive control	Negative control	Protein localization	Positivity in other species
Frozen tissue and cytospins									
Recommended	NAT105	1:4	supernatant	None	goat anti mouse HRP DAKO	Tonsil		membrane	
Paraffin tissue									

Recommended	NAT105	1:50	purified 1mg/mi	Tris-EDTA	Novolink	Tonsil		membrane	
Immunofluorescence									
Recommended	NAT105	1:30	purified 1mg/ml	Tris-EDTA	Goat anti mouse IgG1 Alexa	Tonsil		membrane	

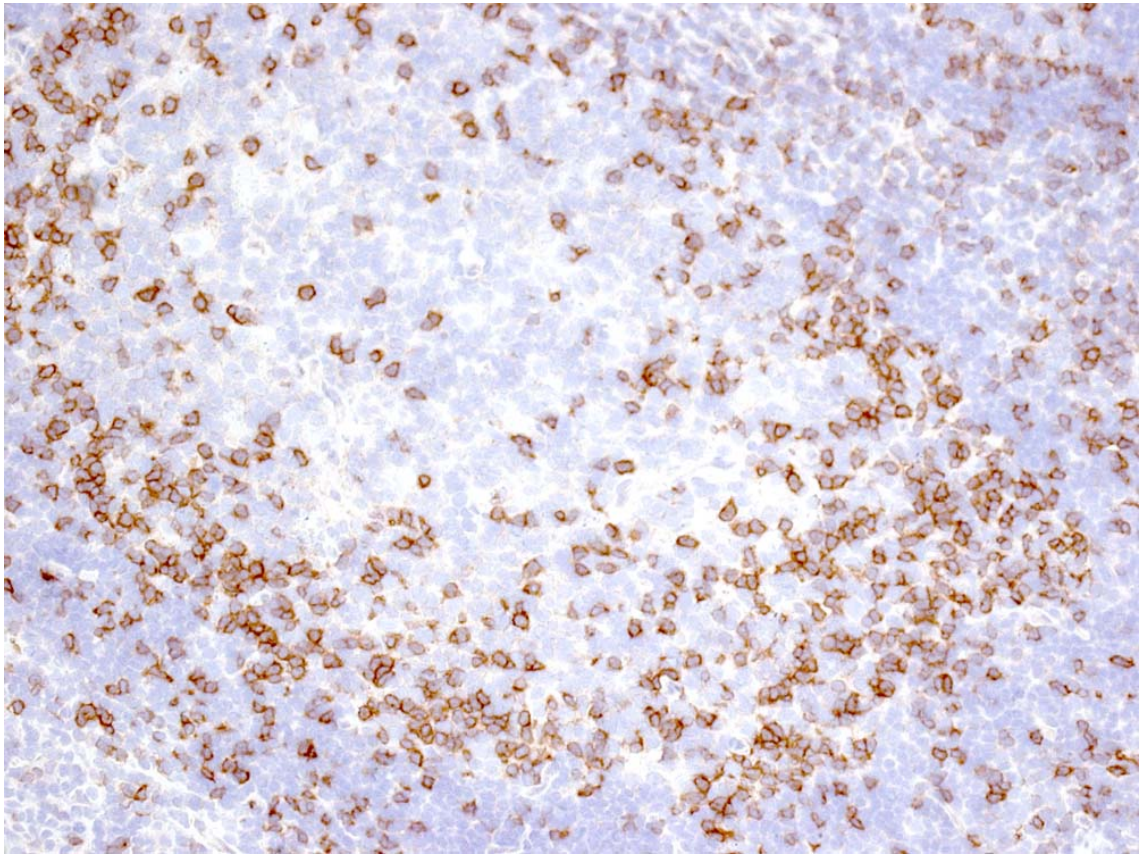


Anti-PD1 antibodies (NAT105 and R&D polyclonal) in HEK transfected cells.

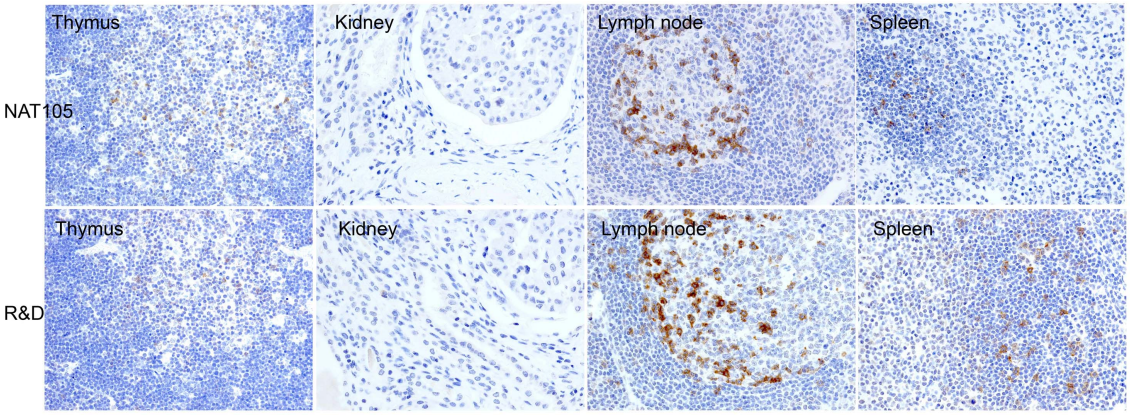
Validation of NAT105 monoclonal antibody in transfected cells. Hek-BTLA transfected cells were used as negative control.



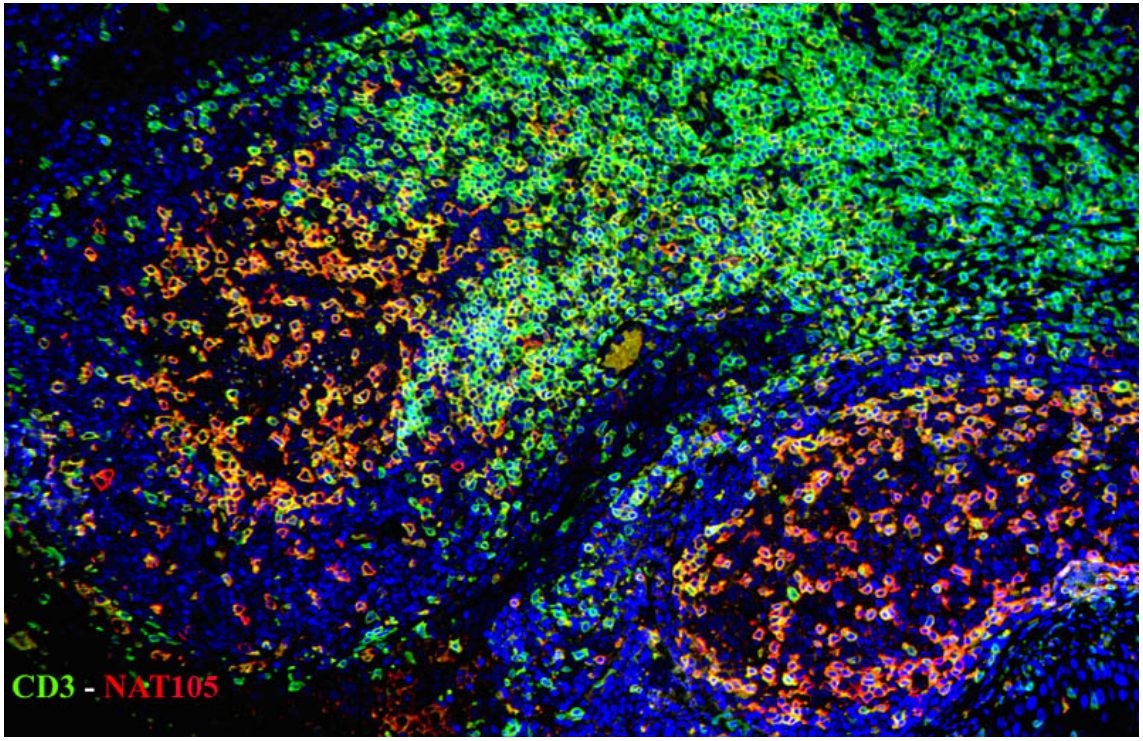
PD1 (NAT105 and R&D antibodies) expression in YT cell line.



Anti-PD1 (NAT105) antibody on frozen tonsil section.



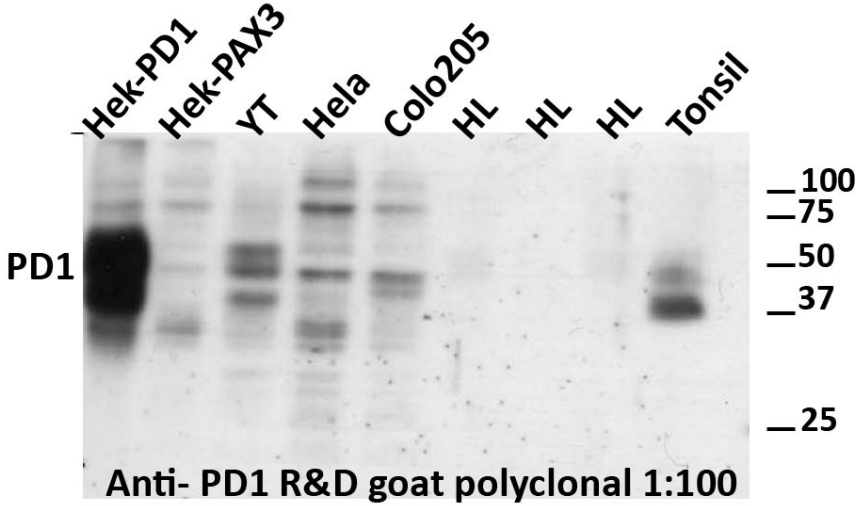
PD1 (NAT105 and R&D) expression on human paraffin sections

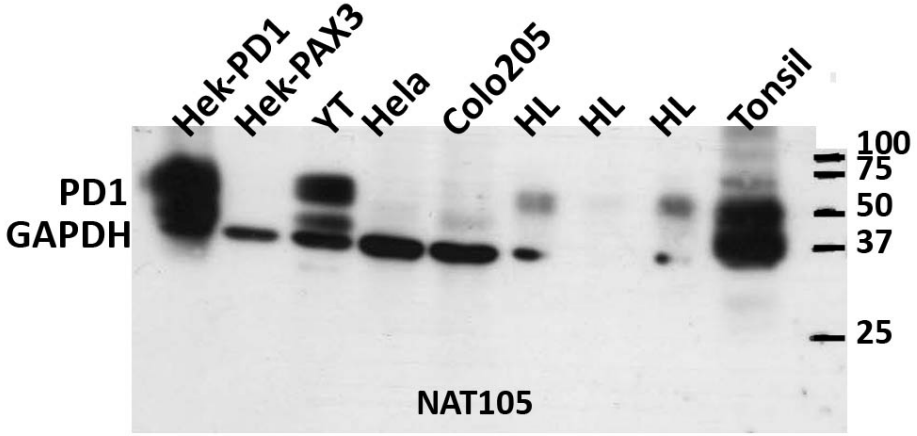


Expression of PD1 (NAT105) antigen by lymphoid cells.

Double immunofluorescent staining shows that many of the PD1 (NAT105)-positive cells (red) in the germinal center cells co-express CD3 (green).

WB Techniques	Clone	Dilution	Antibody concentration	Positive control	Negative control	Expected MW	Observed Mw	Positivity in other species
Western Blotting								
Recommended	NAT105	1:2	supernatant	YT cell line	Colo 205 cell line	32 KDa	47 kDa	
Immunoprecipitation								
Recommended	NAT105		supernatant	YT cell line	Ramos cell line	32KDa	47KDa	





WB of anti-PD1 (NAT105 and R&D) antibodies.

Both antibodies are dettecting a 47kDa band.

Anti GAPDH was used as loading control.

Lane 1 Hek-PD1 transfected cells (20ug) (+)

Lane 2 Hek-PAX3 transfected cells (20ug) (-)

Lane 3 YT cell line (100ug) (+)

Lane 4 Hela cell line (100ug) (-)

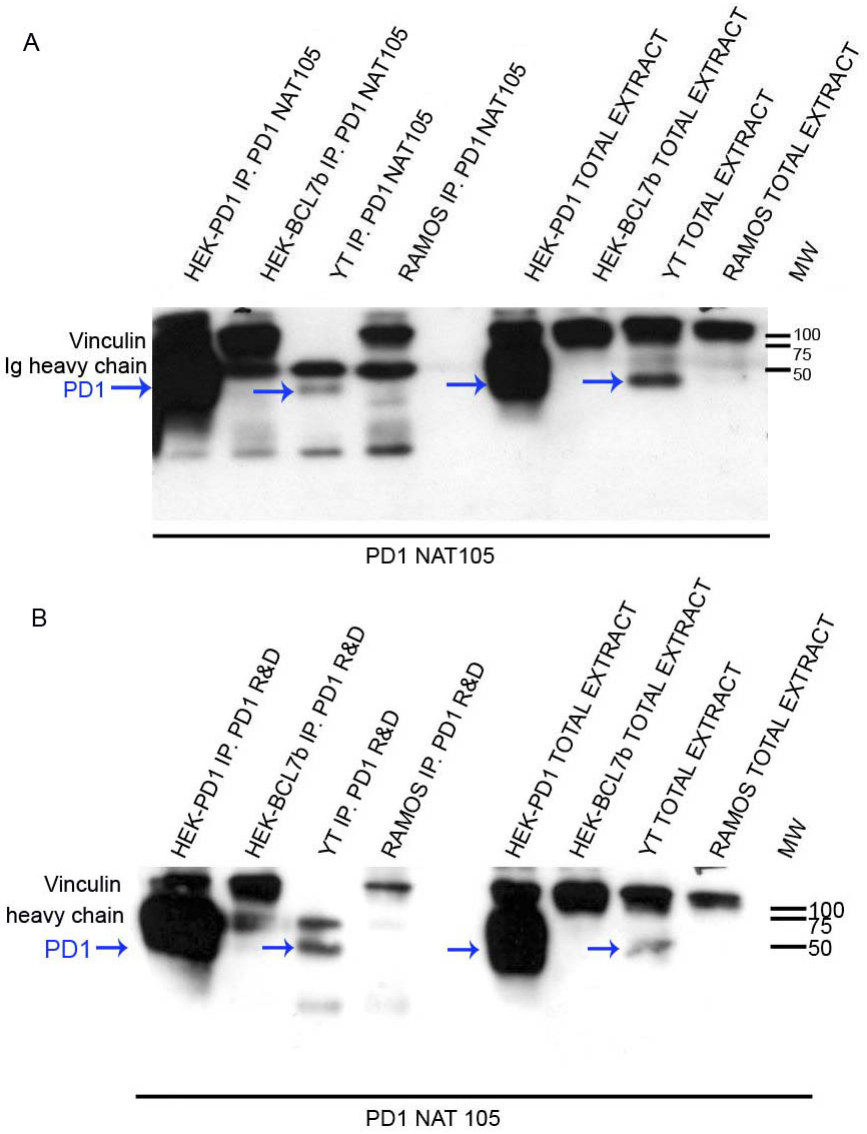
Lane 5 Colo205 cell line (100ug) (-)

Lane 6 Human HL (100ug) (+)

Lane 7 Human HL (100ug) (+)

Lane 8 Human HL (100ug) (+)

Lane 9 Human tonsil (100ug) (+)

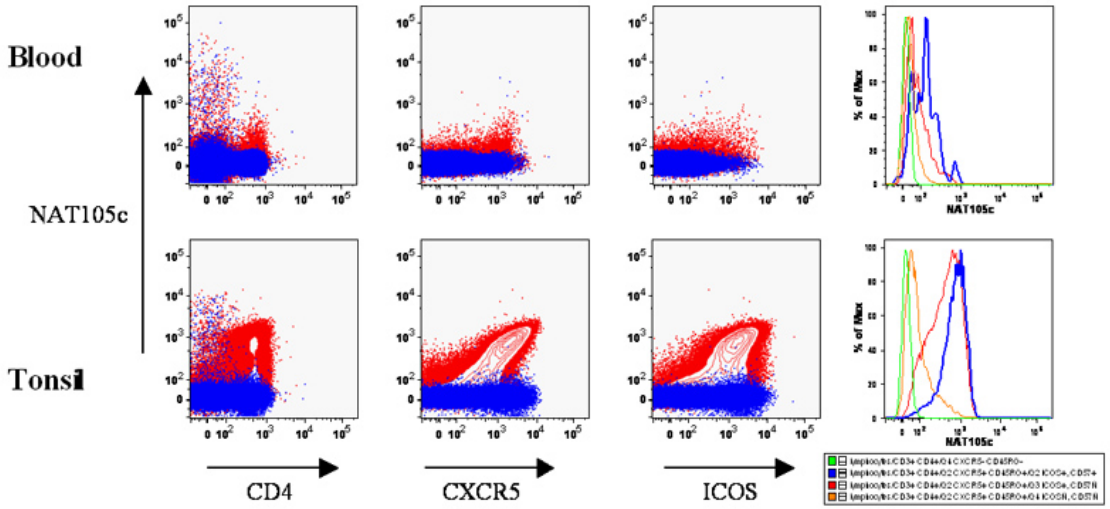


NAT105 mAb can be use in IP technique

A. Immunoprecipitation of protein extracts from HEK-PD1, HEK-Bcl7b, YT and Ramos cell lines with NAT105C mAb (1ul/lane) followed by western blotting with the same antibody (neat supernatant).

B. Immunoprecipitation of protein extracts from HEK-PD1, HEK-Bcl7b, YT and Ramos cell lines with goat R&D antibody (1ul/lane) followed by western blotting with NAT105 antibody (neat supernatant).

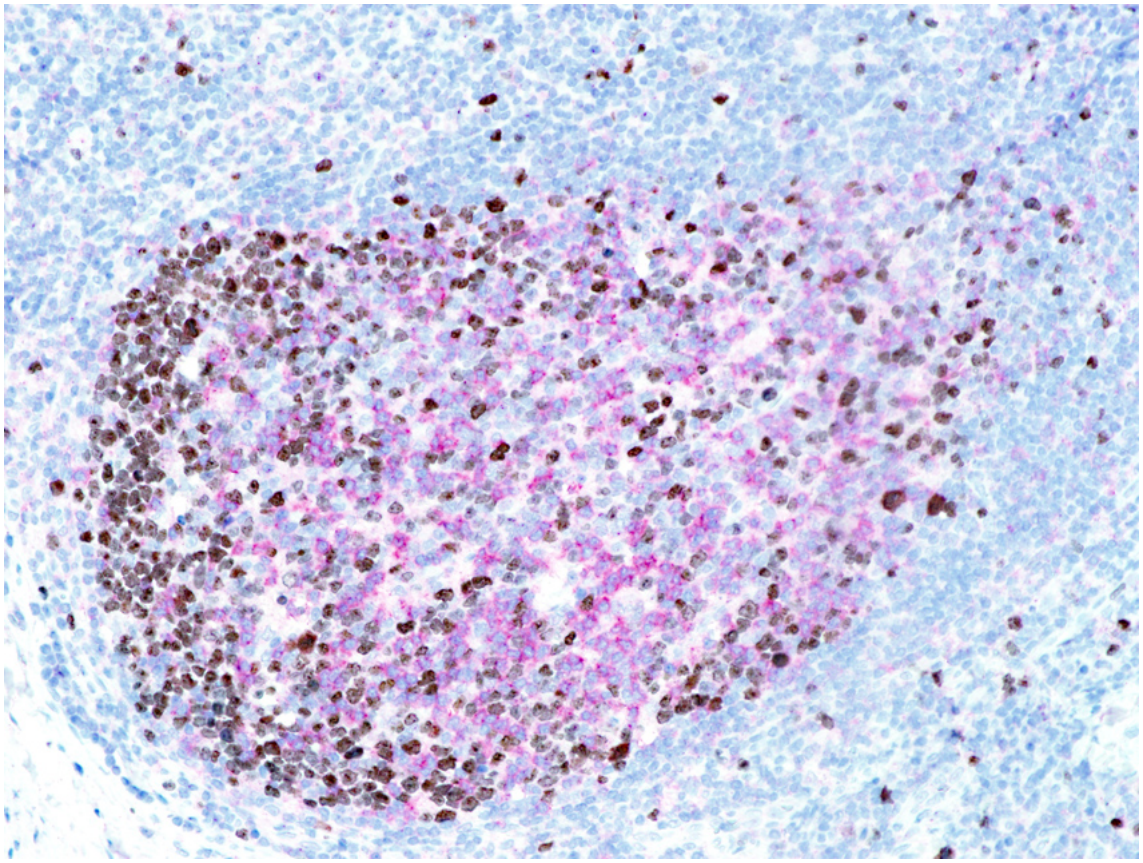
FLOW CYTOMETRY	Clone	Dilution	Positive control	Negative control	Type of fluorocrom
Recommended	NAT	1:20 purified 1mg/ml	Tonsil		



NAT105 staining in blood and tonsillar lymphocytes

Column one. NAT105 (using, as second step, anti-mouse IgG1-PE) and CD4 staining in lymphocytes. Overlaid blue indicates staining in a parallel well with CD4 and anti-mouse iIg1-Pe in the absence of NAT105 (negative control). Column two. NAT105 and CXCR5 staining in CD3+ CD4+ lymphocytes. Column three. NAT105 and ICOS (PerCP) staining in CD3+ CD4+ Lymphocytes. Column four. Overlaid histograms of NAT105 staining in ICOS+CD57+ (blue line), ICOS+ CD57-(red line) and ICOS-CD57- (brown line) CXCR5+ CD45RO+CD4+ lymphocytes, and CXCR5- CD45RO- CD4+ lymphocytes (green line)

OTHERS	Title	Description
Recommended	Double PD1/KI67 immunoenzimatic staining	Double immunoenzymatic labeling of p araffin sections was performed using a standard BOND system protocol with the Abs PD-1/KI67 (Leica, Milton Ke)



Double PD1/KI67 immunoenzimatic staining

Double immunoenzymatic staining shows the expression of PD1 (red) and KI67 (nuclear, brown) and that PD1 cells lack the proliferation marker Ki-67.