

PathPlex® Panel 7

(CD8 alpha, Cytokeratin, CD45RO, CD4, FOXP3)



Catalog No. A810-007

CONTENTS	A700-044	Rabbit anti-CD8 alpha Recombinant Monoclonal Antibody [BLR044F]
	A500-019A	Mouse anti-Cytokeratin Monoclonal Antibody, Purified [AE1/AE3]
	A500-020A	Mouse anti-CD45RO Monoclonal Antibody, Purified [UCHL-1]
	A700-015	Rabbit anti-CD4 Recombinant Monoclonal Antibody [BL-155-1C11]
	A700-034	Rabbit anti-FOXP3 Recombinant Monoclonal Antibody [BLR034F]

APPLICATIONS IHC-IF, mIF

SPECIES REACTIVITY Human

AMOUNT 1 Panel

STORAGE/SHELF LIFE 2 - 8° C / 1 year from date of receipt

PHYSICAL STATE Liquid

ORIGIN USA

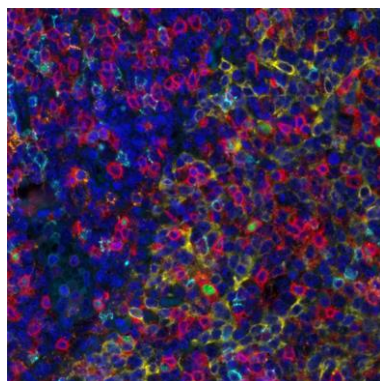
ADDITIONAL INFO <https://www.bethyl.com/product/A810-007>

Use the link above to view SDS, a current list of citations, and other product specific information.

Catalog Number	Target	Dilution	Order of Staining	Opal Fluorophore
A700-044	CD8 alpha	1:250	1	480
A500-019A	Cytokeratin	1:250	2	570
A500-020A	CD45RO	1:250	3	690
A700-015	CD4	1:250	4	620
A700-034	FOXP3	1:250	5	520

The table above provides initial conditions. Further optimization with the choice tissue and balance of tryamide detection signal should be performed. Please see the Bethyl multiplex protocol for optimization.

<https://www.bethyl.com/content/protocol-multiplexing>



Detection of human CD4 (orange), CD8 (cyan), CD45RO (red), cytokeratin (yellow), and FOXP3 (green) in FFPE tonsil by IHC-IF. Rabbit anti-CD4 recombinant monoclonal [BL-155-1C11] (A700-015), rabbit anti-CD8 alpha recombinant monoclonal [BLR044F] (A700-044), mouse anti-CD45RO monoclonal [UCHL-1] (A500-020A), mouse anti-cytokeratin monoclonal [AE1/AE3] (A500-019A) and rabbit anti-FOXP3 recombinant monoclonal [BLR034F] (A700-034). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P) and HRP-conjugated goat anti-mouse IgG (A90-116P). Substrate: Opal™ 480, 520, 570, 620, 690 and 780. Counterstain: DAPI (blue).

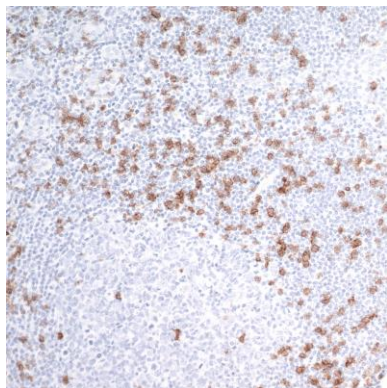
CD8 alpha Recombinant Monoclonal Antibody [BLR044F]

Rabbit Recombinant Monoclonal

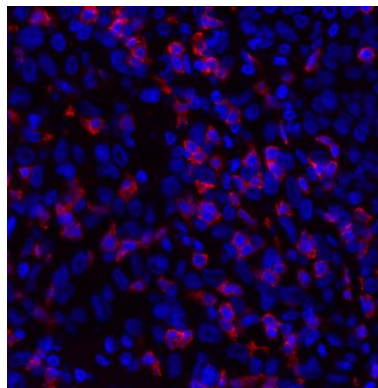
Purified Protein ID NP_001759.3
Catalog No. A700-044 GeneID 925



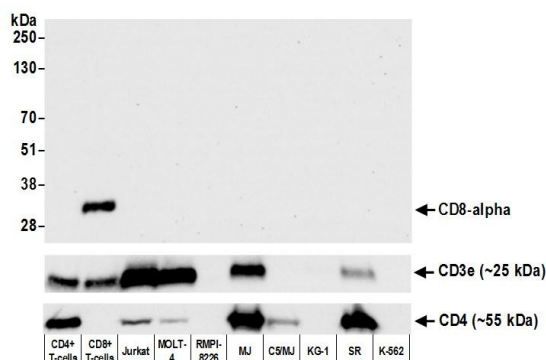
APPLICATIONS	WB, IHC, IHC-IF, mIF
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	250 µg/ml
STORAGE/SHELF LIFE	2 – 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BLR044F
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant. Immunogen was a peptide representing a region between residue 185 and the C-terminus (residue 235) of human T-cell surface glycoprotein CD8 alpha chain using the numbering given in entry NP_001759.3 (Gene ID 925)
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use. Western Blot 1:1000 Immunohistochemistry 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections. Immunofluorescence (IHC) 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections. Multiplex Immunofluorescence 1:250
IHC HUMAN CONTROLS	Melanoma, Tonsil
ADDITIONAL INFO	https://www.bethyl.com/product/A700-044 Use the link above to view SDS, a current list of citations, and other product specific information.



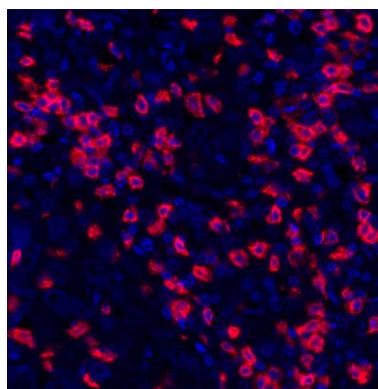
Detection of human CD8 alpha by immunohistochemistry. *Sample:* FFPE section of human tonsil. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



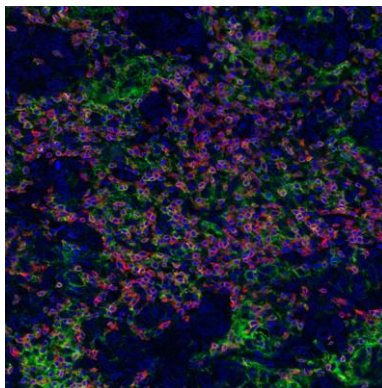
Detection of human CD8 alpha by immunohistochemistry. *Sample:* FFPE section of human melanoma. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:100. *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-101D4).



Detection of human CD8 alpha by western blot. *Samples:* Whole cell lysate (50 µg) from CD4+ T-cells, CD8+ T-cells, Jurkat, MOLT-4, RPMI-8226, MJ, C5/MJ, KG-1, SR, and K-562 cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 30 seconds. Lower panel: Recombinant monoclonal antibodies to CD3e (A700-016) and CD4 (A700-015).



Detection of human CD8 alpha (red) by immunohistochemistry. *Sample:* FFPE section of human breast carcinoma. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* Opal™. *Counterstain:* DAPI (blue).



Detection of human CD3E (orange), CD8 alpha (red), and PD-L1 (green) in FFPE breast carcinoma by IHC-IF. Rabbit anti-CD3E recombinant monoclonal [BL-298-5D12] (A700-016), rabbit anti-CD8 alpha recombinant monoclonal [BLR044F] (A700-044), rabbit anti-PD-L1 recombinant monoclonal [BLR020E] (A700-020). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P). Substrate: Opal™ 520, 620, and 690. Counterstain: DAPI (blue).

Cytokeratin Monoclonal Antibody [AE1 /AE3]

Mouse Monoclonal

Purified

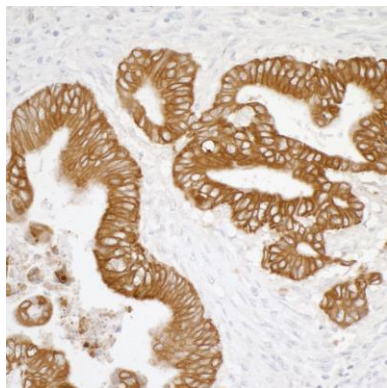
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Catalog No. A500-019A

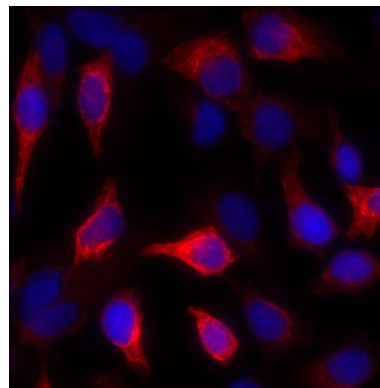
GeneID 3858



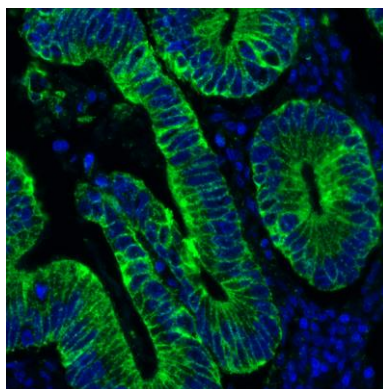
APPLICATIONS	IHC, IHC-IF, ICC-IF
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	200 µg/ml
STORAGE/SHELF LIFE	2 – 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Phosphate Buffered Saline (PBS) containing 0.09% Sodium Azide
ISOTYPE	IgG1
CLONE #	AE1 /AE3
ORIGIN	USA
PRODUCTION PROCEDURES	The antibody was purified from cell culture supernatant. Immunogen: Human epidermal callus
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use. Immunohistochemistry 1:100 – 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections. Immunofluorescence (IHC) 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections. Immunofluorescence (ICC) 1:100 – 1:500
IHC HUMAN CONTROLS	Colon Carcinoma, Prostate Carcinoma
ADDITIONAL INFO	https://www.bethyl.com/product/A500-019A Use the link above to view SDS, a current list of citations, and other product specific information.



Detection of human cytokeratin by immunohistochemistry. *Sample:* FFPE section of human colon carcinoma. *Antibody:* Mouse monoclonal anti-Cytokeratin antibody [AE1/AE3] (A500-019A Lot 1) used at a dilution of 1:250. *Secondary:* HRP-conjugated goat anti-mouse IgG (A90-116P). *Substrate:* DAB.



Detection of human Cytokeratin by immunocytochemistry. *Sample:* acetone/methanol fixed Hela cells. *Antibody:* Mouse monoclonal anti-Cytokeratin antibody [AE1/AE1] (A500-019A lot 1) used at 1:100. *Secondary:* DyLight® 594-conjugated goat anti-mouse IgG (A90-116D4). *Counterstain:* DAPI.



Detection of human Cytokeratin (green) by immunohistochemistry. *Sample:* FFPE section of human colon carcinoma. *Antibody:* Mouse anti-Cytokeratin monoclonal antibody [AE1/AE3] (A500-019A lot 1) used at 1:500. *Secondary:* HRP-conjugated goat anti-mouse IgG (A90-116P). *Substrate:* Opal™. *Counterstain:* DAPI (blue).

CD45RO Monoclonal Antibody [UCHL-1]

Mouse Monoclonal

Purified

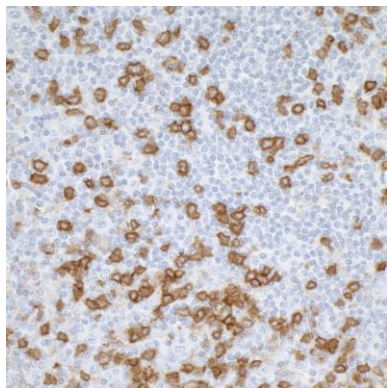
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Catalog No. A500-020A

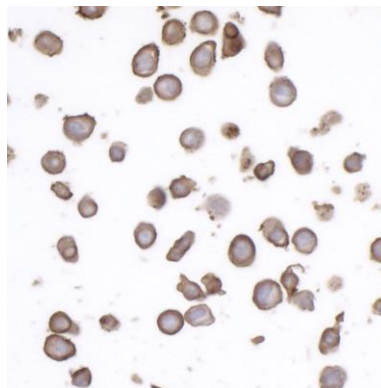
GeneID 5788



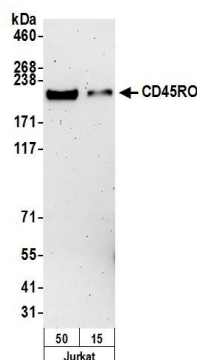
APPLICATIONS	WB, IHC, ICC, IHC-IF
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	50 µg/ml
STORAGE/SHELF LIFE	2 - 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Phosphate Buffered Saline (PBS) containing 0.09% Sodium Azide
ISOTYPE	IgG2a
CLONE #	UCHL-1
ORIGIN	USA
PRODUCTION PROCEDURES	The antibody was purified from cell culture supernatant. Immunogen: Cultured T-cells from an IL-2-dependent T-cell line (CA1)
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use. Western Blot 1:250 Immunohistochemistry 1:100 - 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections. Immunocytochemistry 1:100 - 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE cell sections. Immunofluorescence (IHC) 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.
APPLICATION NOTES	Validation by western blot was performed using a 4-8% SDS-PAGE.
IHC HUMAN CONTROLS	Tonsil, Tumor-infiltrating Lymphocytes
ADDITIONAL INFO	https://www.bethyl.com/product/A500-020A Use the link above to view SDS, a current list of citations, and other product specific information.



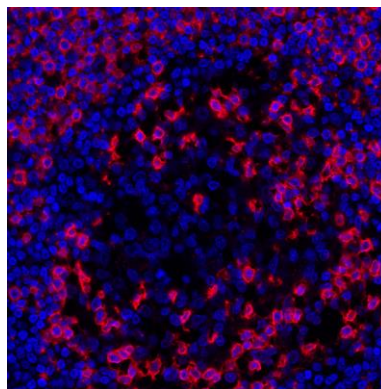
Detection of human CD45RO by immunohistochemistry.
Sample: FFPE section of human tonsil. *Antibody:* Mouse monoclonal anti-CD45RO antibody [UCHL-1] (A500-020A Lot 1) used at a dilution of 1:250. *Secondary:* HRP-conjugated goat anti-mouse IgG (A90-116P). *Substrate:* DAB. *Counterstain:* Hematoxylin (blue).



Detection of human CD45RO by immunocytochemistry.
Sample: FFPE section of human SR (lymphoma) cell line. *Antibody:* mouse monoclonal anti-CD45RO antibody [UCHL-1] (A500-020A Lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-mouse IgG (A90-116P). *Substrate:* DAB.



Detection of human CD45RO by western blot. *Samples:* Whole cell lysate (15 and 50 μ g) from Jurkat cells prepared using NETN lysis buffer. *Antibody:* mouse anti-CD45RO antibody [UCHL-1] (A500-020A Lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-mouse IgG (A90-116P). Chemiluminescence with an exposure time of 3 minutes.



Detection of human CD45RO (red) by immunohistochemistry. *Sample:* FFPE section of human lung carcinoma. *Antibody:* Mouse anti-CD45RO monoclonal antibody [UCHL-1] (A500-020A lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-Mouse IgG (A90-116P). *Substrate:* Opal™. *Counterstain:* DAPI (blue).

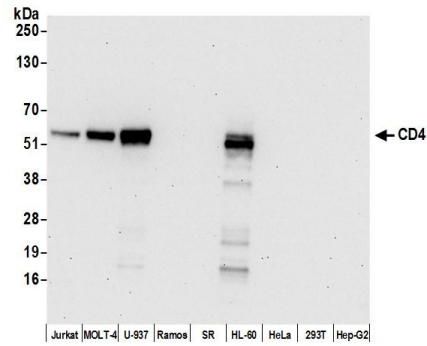
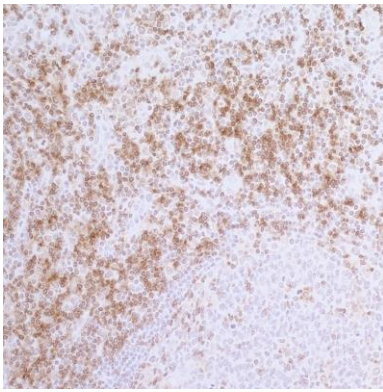
CD4 Recombinant Monoclonal Antibody [BL-155-1C11]

Rabbit Recombinant Monoclonal

Purified Protein ID NP_000607.1
Catalog No. A700-015 GeneID 920



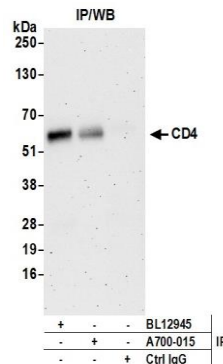
APPLICATIONS	WB, IP, IHC
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	1000 µg/ml
STORAGE/SHELF LIFE	2 – 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Borate Buffered Saline (BBS) pH 8.2 with 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BL-155-1C11
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant. Immunogen was a recombinant protein representing the extracellular domain of human CD4 (residues 26–397, NP_000607.1).
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use. Western Blot 1:1000 Immunoprecipitation 20 µl/mg lysate Immunohistochemistry 1:100 – 1:500. Epitope retrieval with Tris-EDTA pH 9.0 is recommended for FFPE tissue sections.
IHC HUMAN CONTROLS	Appendix, Spleen, Tonsil
ADDITIONAL INFO	https://www.bethyl.com/product/A700-015 Use the link above to view SDS, a current list of citations, and other product specific information.



Detection of human CD4 by immunohistochemistry.

Sample: FFPE section of human appendix. *Antibody:* Rabbit anti-CD4 recombinant monoclonal antibody [BL-155-1C11] (A700-015 lot 1) used at 1:200. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). *Substrate:* DAB.

Detection of human CD4 by western blot. *Samples:* Whole cell lysate (15 µg) from Jurkat, MOLT-4, U-937, Ramos, SR, HL-60, HeLa, HEK293T, and Hep-G2 cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-CD4 recombinant monoclonal antibody [BL-155-1C11] (A700-015 lot 1) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 30 seconds.



Detection of human CD4 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1 mg for IP; 20% of IP loaded) from MOLT-4 cells prepared using NETN lysis buffer. *Antibodies:* Rabbit anti-CD4 recombinant monoclonal antibody [BL-155-1C11] (A700-015 lot 1) used for IP at 20 µl per reaction. CD4 was also immunoprecipitated by rabbit anti-CD4 antibody (BL12945). For blotting immunoprecipitated CD4, A700-015 was used at 1:1000. Chemiluminescence with an exposure time of 3 minutes.

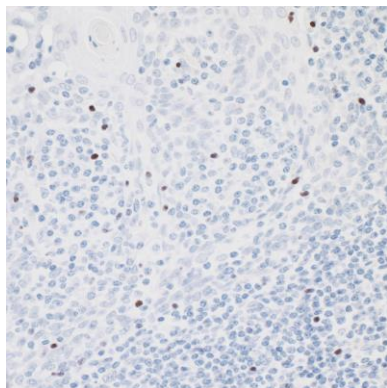
FOXP3 Recombinant Monoclonal Antibody [BLR034F]

Rabbit Recombinant Monoclonal

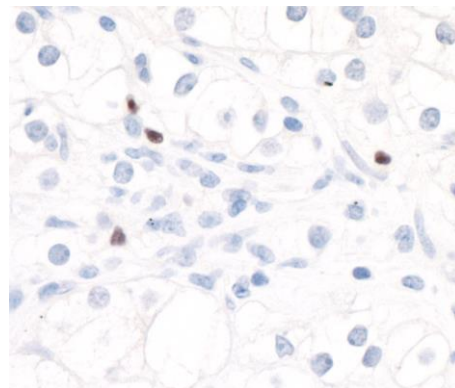
Purified Protein ID NP_054728.2
Catalog No. A700-034 GeneID 50943



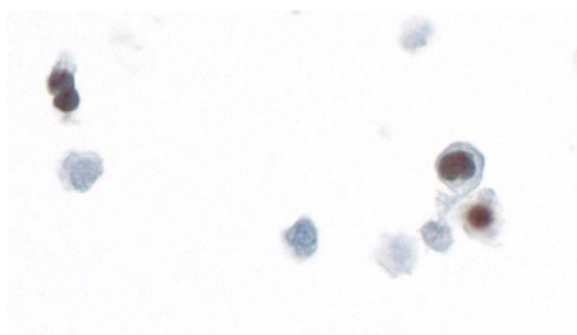
APPLICATIONS	WB, IP, IHC, ICC, IHC-IF
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	1000 µg/ml
STORAGE/SHELF LIFE	2 – 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Borate Buffered Saline (BBS) pH 8.2 with 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BLR034F
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant. Immunogen was a peptide representing a region between residue 275 and 325 of human Forkhead box P3 using the numbering given in entry NP_054728.2 (Gene ID 50943).
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use. Western Blot 1:1000 Immunoprecipitation 6 ul/1mg lysate Immunohistochemistry 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections. Immunocytochemistry 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections. Immunofluorescence (IHC) 1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.
IHC HUMAN CONTROLS	Breast Carcinoma, Lung Carcinoma, Prostate Carcinoma, Renal Cell Carcinoma, Tonsil, MJ Cells
ADDITIONAL INFO	https://www.bethyl.com/product/A700-034 Use the link above to view SDS, a current list of citations, and other product specific information.



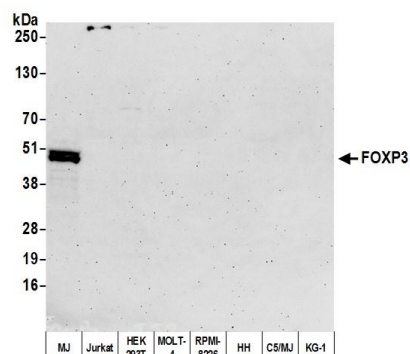
Detection of human FOXP3 by immunohistochemistry.
Sample: FFPE section of human tonsil. *Antibody:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034-1) used at 1:200. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



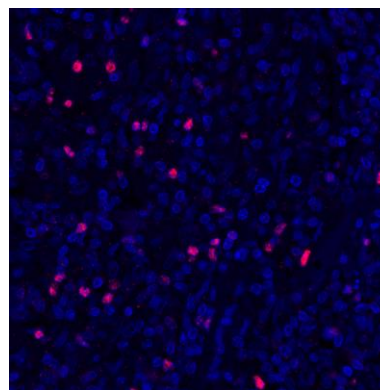
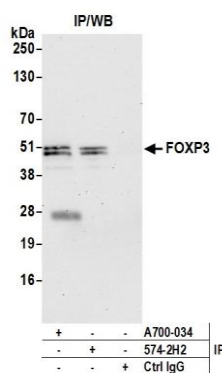
Detection of human FOXP3 by immunohistochemistry.
Sample: FFPE section of human renal cell carcinoma. *Antibody:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034-1) used at 1:200. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



Detection of human FOXP3 by immunocytochemistry.
Sample: FFPE section of MJ cells. *Antibody:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034-1) used at 1:200. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



Detection of human FOXP3 by western blot. *Samples:* Whole cell lysate (50 µg) from MJ, Jurkat, HEK293T, MOLT-4, RPMI-8226, HH, C5/MJ, and KG-1 cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034 lot 1) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 3 minutes.



Detection of human FOXP3 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from MJ cells prepared using NETN lysis buffer. *Antibodies:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034 lot 1) used for IP at 6 μ l per reaction. FOXP3 was also immunoprecipitated by rabbit anti-FOXP3 antibody [574-2H2]. For blotting immunoprecipitated FOXP3, A700-034 was used at 1:1000. Chemiluminescence with an exposure time of 75 seconds.

Detection of human FOXP3 (red) by immunohistochemistry. *Sample:* FFPE section of human breast carcinoma. *Antibody:* Rabbit anti-FOXP3 recombinant monoclonal antibody [BLR034F] (A700-034 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* Opal™. *Counterstain:* DAPI (blue).