

PathPlex® Panel 1 (CD3E, CD8 alpha, PD-L1)



Catalog No. A810-001

CONTENTS	A700-016	Rabbit anti-CD3E Recombinant Monoclonal Antibody [BL-298-5D12]
	A700-044	Rabbit anti-CD8 alpha Recombinant Monoclonal Antibody [BLR044F]
	A700-020	Rabbit anti-PD-L1 Recombinant Monoclonal Antibody [BLR020E]

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

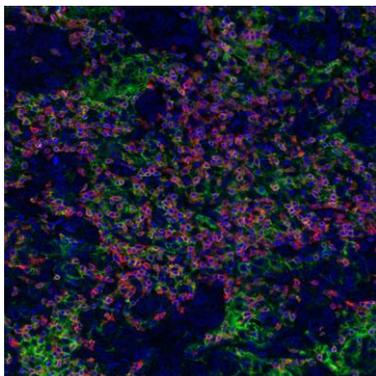
ADDITIONAL INFO <https://www.bethyl.com/product/A810-001>

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
A700-016	CD3E	1:250	1	620
A700-044	CD8 alpha	1:250	2	520
A700-020	PD-L1	1:250	3	690

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 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).

CD3E Recombinant Monoclonal Antibody [BL-298-5D12]

Rabbit Recombinant Monoclonal

Purified Protein ID NP_000724.1
Catalog No. A700-016 GeneID 916



APPLICATIONS	WB, IP, IHC, ICC, IHC-IF, F, mIF
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	Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BL-298-5D12
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant.

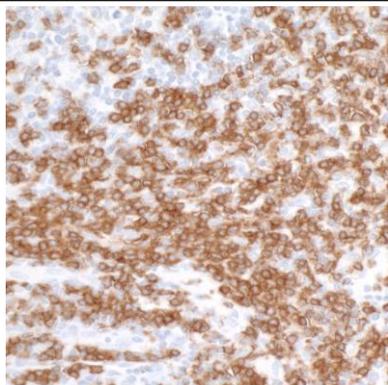
Immunogen was a synthetic peptide representing a region of CD3E and maps to a region between residues 157 to 207 of human CD3e Molecule, Epsilon (CD3-TCR Complex) according to the numbering given in entry NP_000724.1 (GeneID 916).

APPLICATIONS Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.

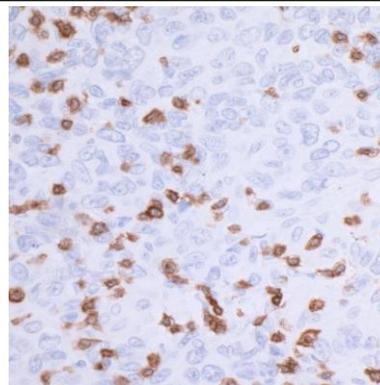
Western Blot	1:1000
Immunoprecipitation	20 µl/mg lysate
Immunohistochemistry	1:100 - 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections.
Immunocytochemistry	1:100 - 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections.
Immunofluorescence (IHC)	1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections.
Flow Cytometry	1 µl per 1 X 10 ⁶ cells
Multiplex Immunofluorescence	1:250

IHC HUMAN CONTROLS Appendix, Metastatic Lymph Node, Tonsil, Jurkat Cells, MOLT-4 Cells

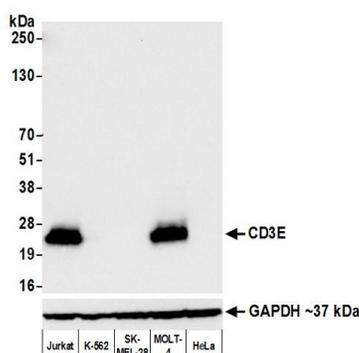
ADDITIONAL INFO <https://www.bethyl.com/product/A700-016>
Use the link above to view SDS, a current list of citations, and other product specific information.



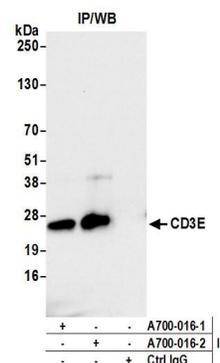
Detection of human CD3E in FFPE tonsil by IHC. *Antibody:* Rabbit anti-CD3E recombinant monoclonal [BL-298-5D12] (A700-016 lot 2). *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



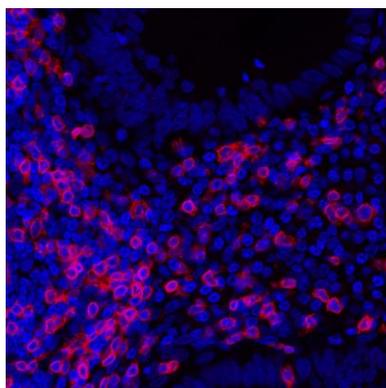
Detection of human CD3E in FFPE breast carcinoma by IHC. *Antibody:* Rabbit anti-CD3E recombinant monoclonal [BL-298-5D12] (A700-016 lot 2). *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.



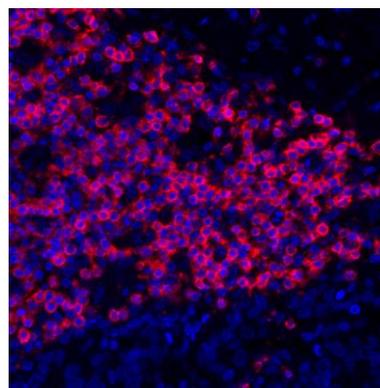
Detection of human CD3E by western blot. *Samples:* Whole cell lysate (50 μ g) from Jurkat, K-562, SK-MEL-28, MOLT-4, and HeLa cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-CD3E recombinant monoclonal antibody [BL-298-5D12] (A700-016 lot 2) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 3 seconds. Lower Panel: Rabbit anti-GAPDH (A300-639A).



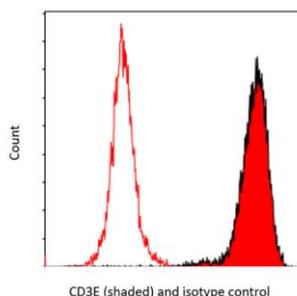
Detection of human CD3E by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from Jurkat cells prepared using NETN lysis buffer. *Antibodies:* Rabbit anti-CD3E recombinant monoclonal antibody [BL-298-5D12] (A700-016 lot 2) used for IP at 20 μ l/mg lysate. CD3E was also immunoprecipitated by a previous lot of this antibody (lot A700-016-1). For blotting immunoprecipitated CD3E, A700-016 was used at 1:1000. Chemiluminescence with an exposure time of 3 seconds.

**Detection of human CD3E by immunohistochemistry.**

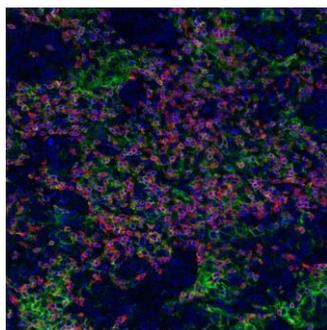
Sample: FFPE section of appendix. *Antibody:* Rabbit anti-CD3E recombinant monoclonal antibody [BL-298-5D12] (A700-016 lot 1) used at 1:250. *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-101D4).

**Detection of human CD3E (red) by immunohistochemistry.**

Sample: FFPE section of human lung carcinoma. *Antibody:* Rabbit anti-CD3E recombinant monoclonal antibody [BL-298-5D12] (A700-016 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* Opal™. *Counterstain:* DAPI (blue).



Detection of human CD3E by flow cytometry. *Sample:* Jurkat cells were fixed in 4% formaldehyde and permeabilized with 90% methanol. *Antibody:* 1E6 cells were incubated with 1 ul of rabbit anti-CD3E recombinant monoclonal antibody [BL-298-5D12] (A700-016 lot 1) (red shaded) or isotype control (unshaded). *Secondary:* DyLight® 650-conjugated goat anti-rabbit IgG (A120-201D5).



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).

CD8 alpha Recombinant Monoclonal Antibody [BLR044F]

Rabbit Recombinant Monoclonal

Purified

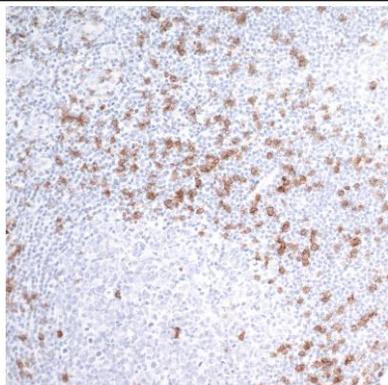
Protein ID NP_001759.3

Catalog No. A700-044

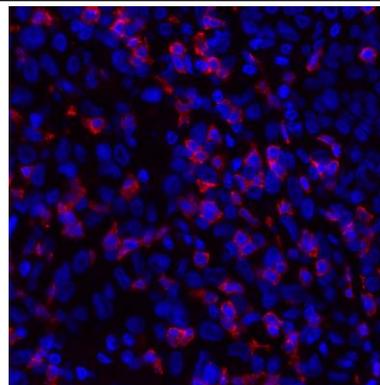
GeneID 925



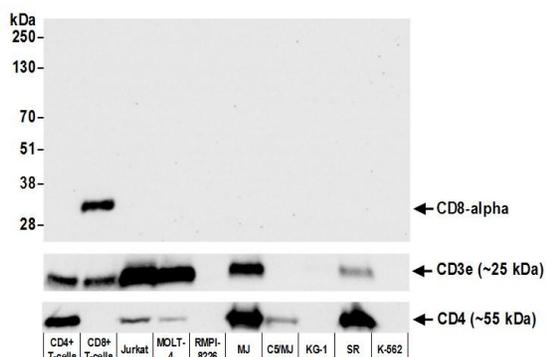
APPLICATIONS	WB, IHC, IHC-IF, mIF
SPECIES REACTIVITY	Human
AMOUNT	100 µl (50+ tests)
CONCENTRATION	250 µg/ml
STORAGE/SHELF LIFE	2 - 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BLR044F
ORIGIN	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.**

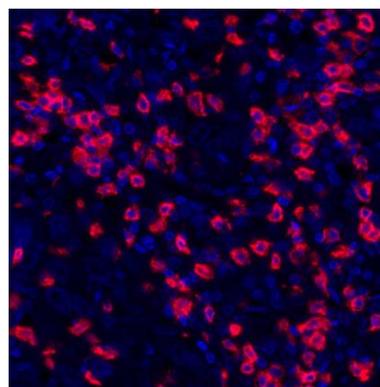
Sample: FFPE section of human tonsil. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P). *Substrate:* DAB.

**Detection of human CD8 alpha by immunohistochemistry.**

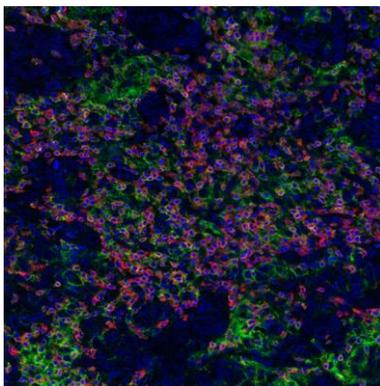
Sample: FFPE section of human melanoma. *Antibody:* Rabbit anti-CD8 alpha recombinant monoclonal antibody [BLR044F] (A700-044 lot 1) used at 1:100. *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-101D4).



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).

PD-L1 Recombinant Monoclonal Antibody [BLR020E]

Rabbit Recombinant Monoclonal

Purified Protein ID NP_054862.1
Catalog No. A700-020 GeneID 29126



APPLICATIONS	WB, IP, IHC, IHC-IF, ICC-IF, mIF
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	Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BLR020E
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant.

Immunogen was a synthetic peptide representing a region between residue 250 and the C-terminus (residue 290) of human programmed cell death 1 ligand 1 (PD-L1) according to the numbering given in entry NP_054862.1 (GeneID 29126).

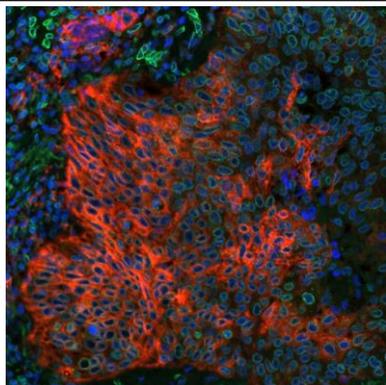
APPLICATIONS Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.

Western Blot	1:1000
Immunoprecipitation	20 µl/mg lysate
Immunohistochemistry	1:100 - 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections.
Immunofluorescence (IHC)	1:100 - 1:500. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections.
Immunofluorescence (ICC)	1:100 - 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections.
Multiplex Immunofluorescence	1:250

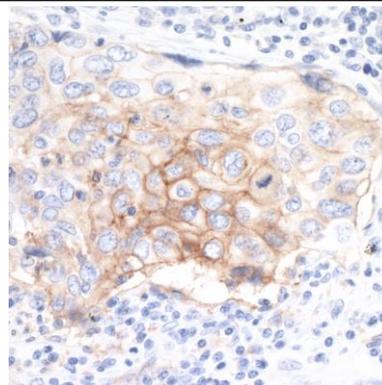
IHC HUMAN CONTROLS Tonsil, HDLM-2 Cells

ADDITIONAL INFO <https://www.bethyl.com/product/A700-020>

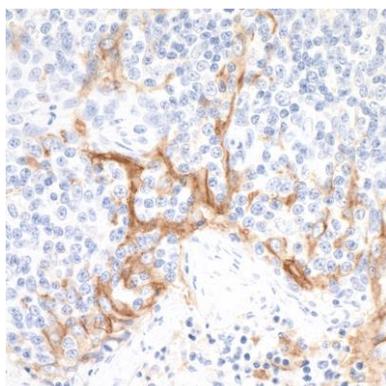
Use the link above to view SDS, a current list of citations, and other product specific information.



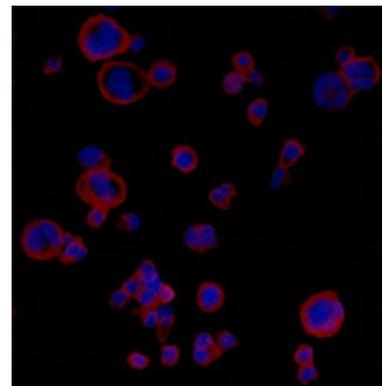
Detection of human PD-L1 (red) and Lamin-A/C (green) in FFPE lung carcinoma by IHC-IF. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal [BLR020E] (A700-020) and rabbit anti-Lamin-A/C (A303-430A). *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-201D4) and DyLight® 488-conjugated goat anti-rabbit IgG (A120-201D2). *Counterstain:* DAPI (blue).



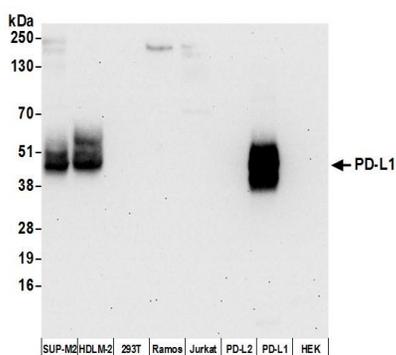
Detection of human PD-L1 by immunohistochemistry. *Sample:* FFPE section of human lung carcinoma. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 lot 1) used at 1:250. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). *Substrate:* DAB.



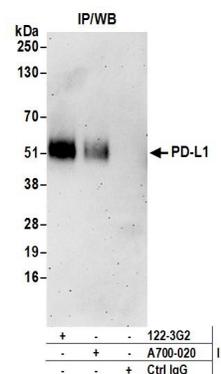
Detection of human PD-L1 by immunohistochemistry. *Sample:* FFPE section of human tonsil. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 lot 1) used at 1:100. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). *Substrate:* DAB.



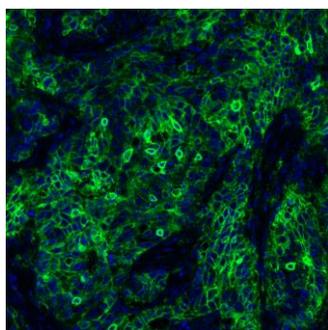
Detection of human PD-L1 by immunocytochemistry. *Sample:* Formaldehyde-fixed HDLM-2 cells. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 lot 1) used at 1:100. *Secondary:* DyLight® 594-conjugated goat anti-rabbit IgG (A120-201D4).



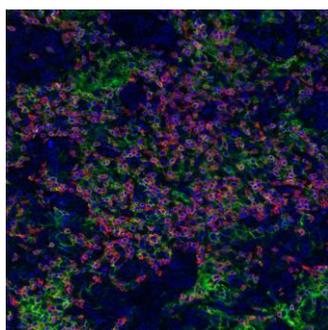
Detection of human PD-L1 by western blot. *Samples:* Whole cell lysate (50 µg) from SUP-M2, HDLM-2, HEK293T, Ramos, Jurkat, PD-L2 OE, PD-L1 OE, and HEK EMPTY cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 lot 1) used for WB at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 30 seconds.



Detection of human PD-L1 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1 mg per IP reaction; 20% of IP loaded) from SUP-M2 cells prepared using NETN lysis buffer. *Antibodies:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 lot 1) used for IP at 20 µl per reaction. PD-L1 was also immunoprecipitated by rabbit anti-PD-L1 antibody [122-3G2]. For blotting immunoprecipitated PD-L1, A700-020 was used at 1:1000. Chemiluminescence with an exposure time of 3 minutes.



Detection of human PD-L1 (green) by immunohistochemistry. *Sample:* FFPE section of human lung carcinoma. *Antibody:* Rabbit anti-PD-L1 recombinant monoclonal antibody [BLR020E] (A700-020 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).