VistaPlex™ Spatial Immune Profiling Assay Kit

CellScape™ Multiplexed Assay Kit for Human FFPE Samples

AbKT-3001-10RXN

Overview

Description

VistaPlex Assay Kits contain ready-to-use, reliable reagents and optimized protocols enabling researchers to obtain quick, robust data with the CellScape platform. The Spatial Immune Profiling Kit enables spatial phenotyping of key immune populations and epithelial cells from human formaldehyde-fixed, paraffin-embedded (FFPE) samples.

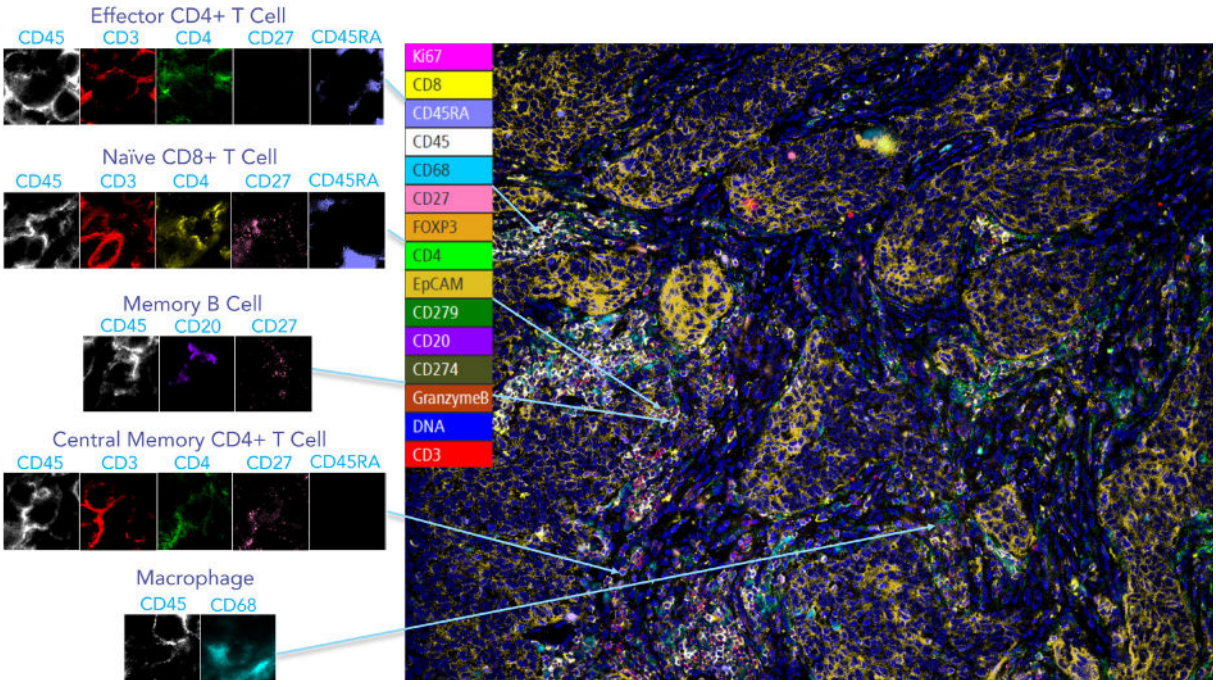
Each Spatial Immune Profiling Assay Kit contains 14 pre-validated fluorescent antibodies at optimized concentrations, a nuclear counterstain, and buffers for staining 10 samples. This kit was validated on lung, breast, and colon tissues, and has also been tested on ovary, spleen, and tonsil tissues. Multiplex assay kit validation is a multi-stage, iterative process to evaluate antibodies for suitability, specificity, and reproducibility.

Report Contents

- Product Details
- Staining Protocol and Gating Strategy
- Assay Validation Process
- Representative Data

Data Summary

Tissue Type	Suitability	Specificity	Reproducibility
Breast FFPE	✓	✓	✓
Colon FFPE	✓	✓	✓
Lung FFPE	✓	✓	✓
Ovary FFPE	✓	✓	Not tested
Spleen FFPE	✓	✓	Not tested
Tonsil FFPE	✓	✓	Not tested



Human FFPE lung cancer tissue section was stained with the Spatial Immune Profiling Assay Kit and imaged on the CellScape system. The biomarkers are used to phenotype immune cell populations such as those shown here with defining markers displayed.

Product Details

Kit Contents

Description	Volume	Cap Color
Anti-Ki-67 Antibody	500 µL	Red
Anti-CD3 Antibody	500 µL	Red
Anti-CD45 Antibody	500 µL	Orange
Anti-CD8 Antibody	500 µL	Orange
Anti-CD45RA Antibody	500 µL	Orange
Anti-CD20 Antibody	500 µL	Yellow
Anti-CD68 Antibody	500 µL	Yellow
Anti-CD27 Antibody	500 µL	Green
Anti-EpCAM Antibody	500 µL	Blue
Anti-FOXP3 Antibody	500 µL	Blue
Anti-CD4 Antibody	500 µL	Blue
Anti-CD279 Antibody	500 µL	Violet
Anti-CD274 Antibody	500 µL	White
Anti-Granzyme B Antibody	500 µL	White
DNA Stain, Hoescht	500 µL	White
AF488 dye quencher (1 of 3)	500 µL	Orange
AF488 dye quencher (2 of 3)	500 µL	Green
AF488 dye quencher (3 of 3)	500 µL	Violet
Antibody Diluent	50 mL	N/A

Storage

Store assay kit components protected from light at 2–8 °C.

Shelf Life

3 months guaranteed, likely stable up to 2 years.

Antigen Retrieval

Antigen retrieval was performed by incubating samples in CC1 antigen retrieval solution (Ventana) on the low setting of a pressure cooker with a maximum temperature of 106 °C–110 °C for 15 minutes.

System Compatibility

The Spatial Immune Profiling Assay Kit has been optimized for use with the CellScape platform. CellScape supports image exports in 32-bit OME tiff, 16-bit tiff, and 8-bit png formats for use in any analysis software.

Intended Use

Research Use Only, not for use in diagnostic procedures. Intended for human FFPE tissues.

Staining Protocol

Panel Set Up

The staining protocol for the Spatial Immune Profiling Assay Kit is accomplished in 7 cycles. A single master mix is created for each cycle, following dilution instructions in the table below. Complete staining and imaging of the 14 targets in this kit using a 1 cm section takes approximately 24 hours. To customize your panel, add additional cycles using pre-validated antibodies from our biomarker catalog or supplement with fluorescently labeled antibodies from your own inventory.

Imaging

The CellScape's high dynamic range (HDR) imaging technology collects images across a series of exposure times to capture the full range of fluorescence values of each stain, including low-expression biomarkers. Each marker is imaged individually and then overlayed by aligning each channel to a reference channel.

Cycle	Target	Filter Set	Antibody Volume	Diluent Volume	Incubation Time
1	Ki-67	FS560	50 µL	400 µL	60 min
	CD3	FS488	50 µL		
2	CD45	FSPerCP	50 µL	300 µL	60 min
	CD8	FS560	50 µL		
	AF488 quencher	None	50 µL		
	CD45RA	FS395	50 µL		
3	CD20	FS560	50 µL	400 µL	60 min
	CD68	FS488	50 µL		
4	CD27	FS560	50 µL	400 µL	60 min
	AF488 quencher	None	50 µL		
5	EpCAM	FSPerCP	50 µL	350 µL	60 min
	FOXP3	FS560	50 µL		
	CD4	FS488	50 µL		
6	CD279	FS560	50 µL	400 µL	60 min
	AF488 quencher	None	50 µL		
7	CD274	FS560	50 µL	350 µL	60 min
	Granzyme B	FS488	50 µL		
	DNA	FS395 & FS421	50 µL		

Example Gating Strategy

Population	Definition
Nucleated Cells	DNA+
Leukocytes	DNA+ CD45+ EpCAM-
Epithelia	DNA+ CD45- EpCAM+
Macrophages	DNA+ CD45+ EpCAM- CD3- CD20- CD68+
T cells	DNA+ CD45+ EpCAM- CD3+ CD20-
Helper T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8-
Cytotoxic T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+
Regulatory T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- FOXP3+
Proliferating CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- Ki-67+
Exhausted CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- CD279+
Central Memory CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- CD27+ CD45RA-
Naive CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- CD27+ CD45RA+
Effector CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- CD27- CD45RA+
Effector Memory CD4+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4+ CD8- CD27- CD45RA-
GranB+ CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD8+ GranB+
Proliferating CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ Ki-67+
Exhausted CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ CD279+
Central Memory CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ CD27+ CD45RA-
Naive CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ CD27+ CD45RA+
Effector CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ CD27- CD45RA+
Effector Memory CD8+ T cells	DNA+ CD45+ EpCAM- CD3+ CD20- CD4- CD8+ CD27- CD45RA-
B cells	DNA+ CD45+ EpCAM- CD3- CD20+
Memory B cells	DNA+ CD45+ EpCAM- CD3- CD20+ CD27+
Naive B cells	DNA+ CD45+ EpCAM- CD3- CD20+ CD27-

Gating Details

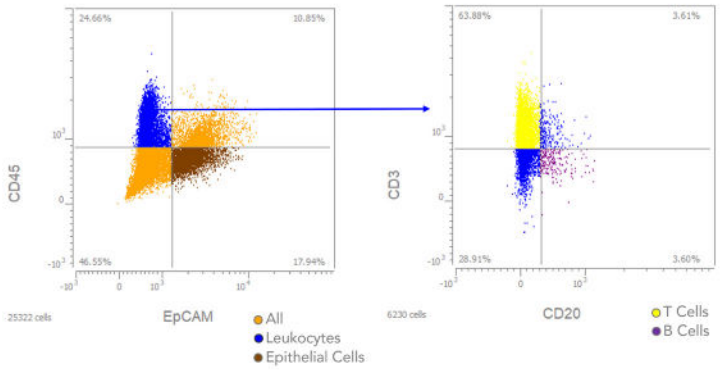
The Spatial Immune Profiling Kit enables spatial phenotyping of key immune populations and epithelial cells, including those listed in the table above. This is a partial list of phenotypes that can be identified with this panel. PD-L1 phenotypes are not represented in this table. Additional phenotypes can be identified based on levels of expression of single markers.

Gating Plots

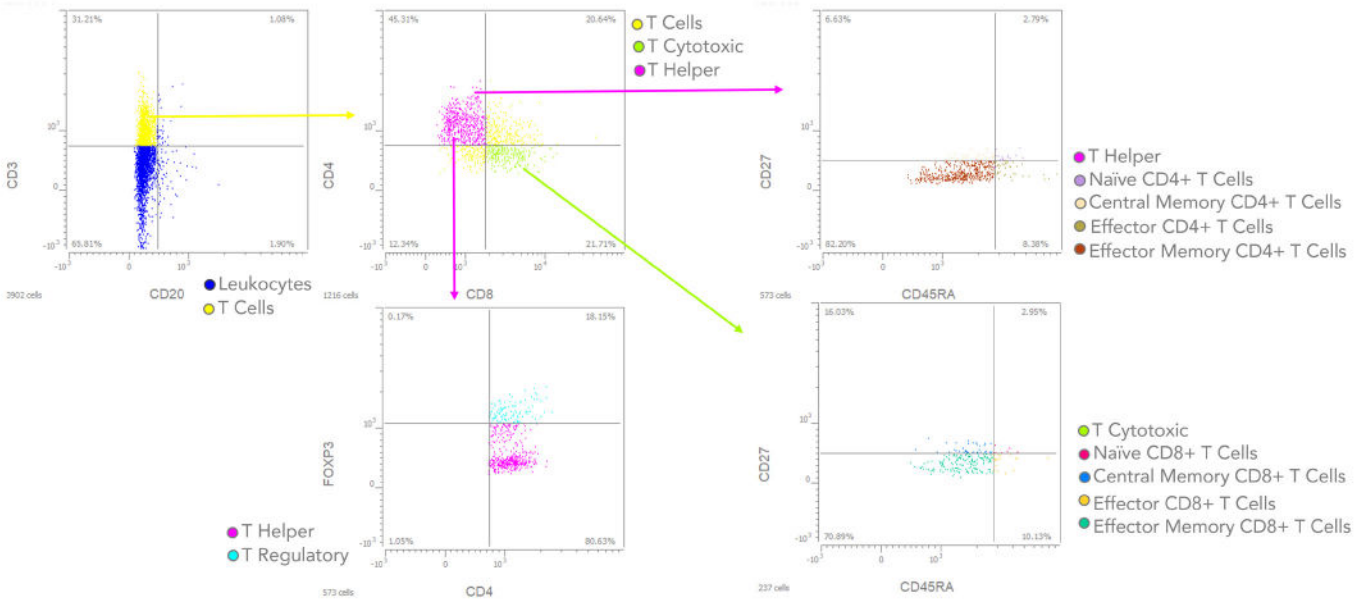
Shown on page 5 are representative bivariate plots of fluorescence intensity, demonstrating a hierarchical gating strategy to characterize and quantify immune cells in human FFPE tissue samples.

Example Gating Strategy (continued)

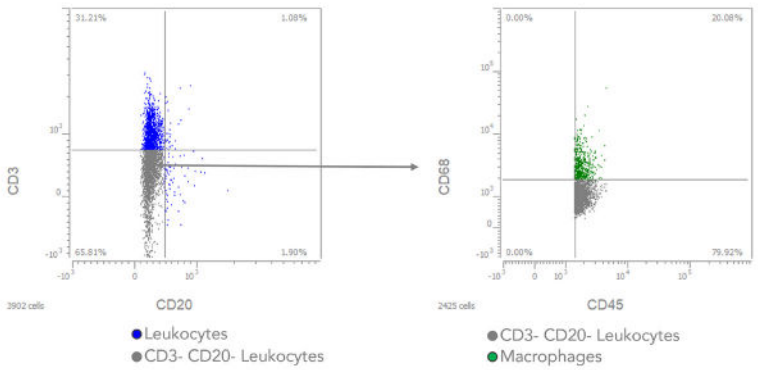
Leukocytes, Epithelial Cells, T Cells, and B Cells



T Cell Subtypes: Helper, Cytotoxic, Regulatory, Naïve, Effector, Central Memory, and Effector Memory



Macrophages



Assay Validation Process

Antibodies in the Spatial Immune Profiling Assay Kit have been fully validated for precise and consistent performance in human FFPE tissue sections.

Suitability

The Spatial Immune Profiling Kit was assessed for suitability by testing all antibodies in the kit in replicates on lung, breast, and colon tissues. Each replicate showed comparable proportions of cell phenotypes to ensure reproducibility. The kit has also been tested successfully on single samples of ovary, spleen, and tonsil tissues.

Specificity

All assay kit antibodies undergo rigorous testing to ensure antibodies bind their intended targets and do not demonstrate off-target effects. Antibodies are initially selected based on reported specificity and fitness for application. The specificity of each antibody is further assessed on the CellScape platform with appropriate counterstains to ensure that antibodies stain their intended tissue structures (e.g., epithelial tissue, stromal region, lymphoid follicles) and localize to the expected subcellular region. The table below lists the expected localization of the biomarker targets in this kit and the antibodies that passed the requirements for staining localization and specificity. A representative composite stain image is shown on page 1.

Reproducibility

The Spatial Immune Profiling Assay Kit was used on three tissue types (breast, colon, and lung), each in duplicate from the same tissue cores. Technical replicates were performed on different days using the same CellScape instrument. Immune cell populations were quantified using the example gating strategy on page 4 and compared across replicates.

Data Analysis

Data analysis was performed in the ZKW Data Wizard application. Cells were identified by computational segmentation on nuclear stain images. Staining data were reviewed independently by two analysts.

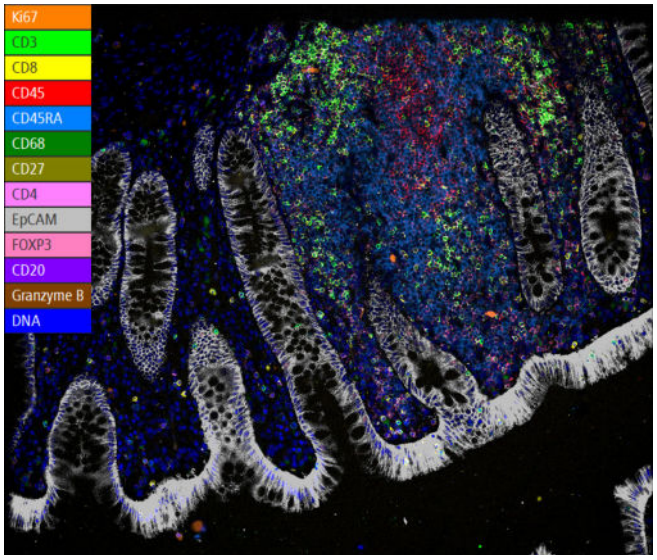
Individual Antibody Validation Results

Marker	Visible Signal	Subcellular Localization	Tissue Localization	Expected Localization	Review by 2 Analysts
Ki-67	Pass	Nucleus	Germinal centers	Pass	Pass
CD3	Pass	Plasma membrane	Lymphoid follicles around germinal centers	Pass	Pass
CD45	Pass	Plasma membrane	Lymphoid infiltrate	Pass	Pass
CD8	Pass	Plasma membrane	Lymphoid follicles around germinal centers	Pass	Pass
CD45RA	Pass	Plasma membrane	Lymphoid infiltrate	Pass	Pass
CD68	Pass	Cytoplasmic membrane	Lymphoid follicles around and in germinal centers	Pass	Pass
CD27	Pass	Plasma membrane	Lymphoid follicles around and in germinal centers	Pass	Pass
EpCAM	Pass	Plasma membrane	Mucosal epithelia	Pass	Pass
FoxP3	Pass	Nucleus	Lymphoid follicles	Pass	Pass
CD4	Pass	Plasma membrane	Lymphoid follicles around germinal centers	Pass	Pass
CD20	Pass	Plasma membrane	Germinal centers	Pass	Pass
CD279	Pass	Plasma membrane	Lymphoid follicles around germinal centers	Pass	Pass
CD274	Pass	Plasma membrane	Tumor epithelia	Pass	Pass
Granzyme B	Pass	Cytoplasmic membrane	Lymphoid follicles around germinal centers	Pass	Pass

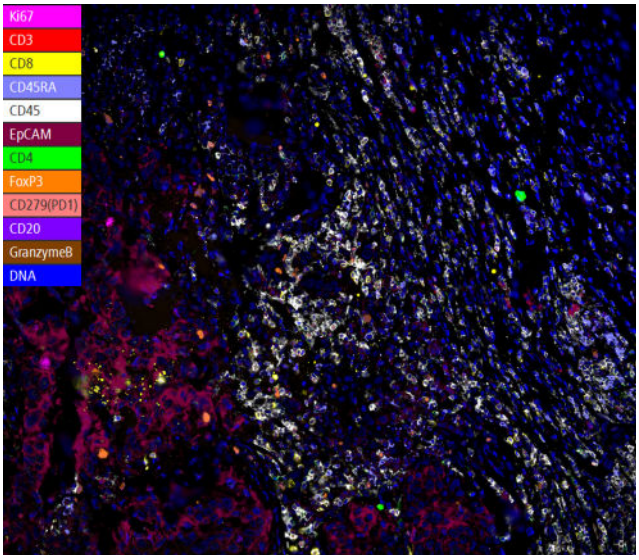
Representative Validation Data

Suitability

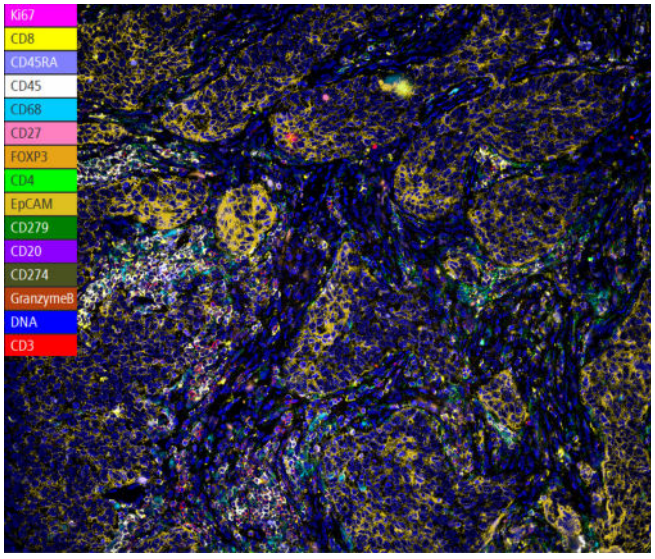
Representative imaging areas of FFPE tissues used for suitability testing are shown. Each section was stained with the Spatial Immune Profiling Assay Kit and imaged on the CellScape instrument. Marker colors are indicated by inset.



Colorectal cancer FFPE tissue with select indicated stains shown.



Breast cancer FFPE tissue with select indicated stains shown.

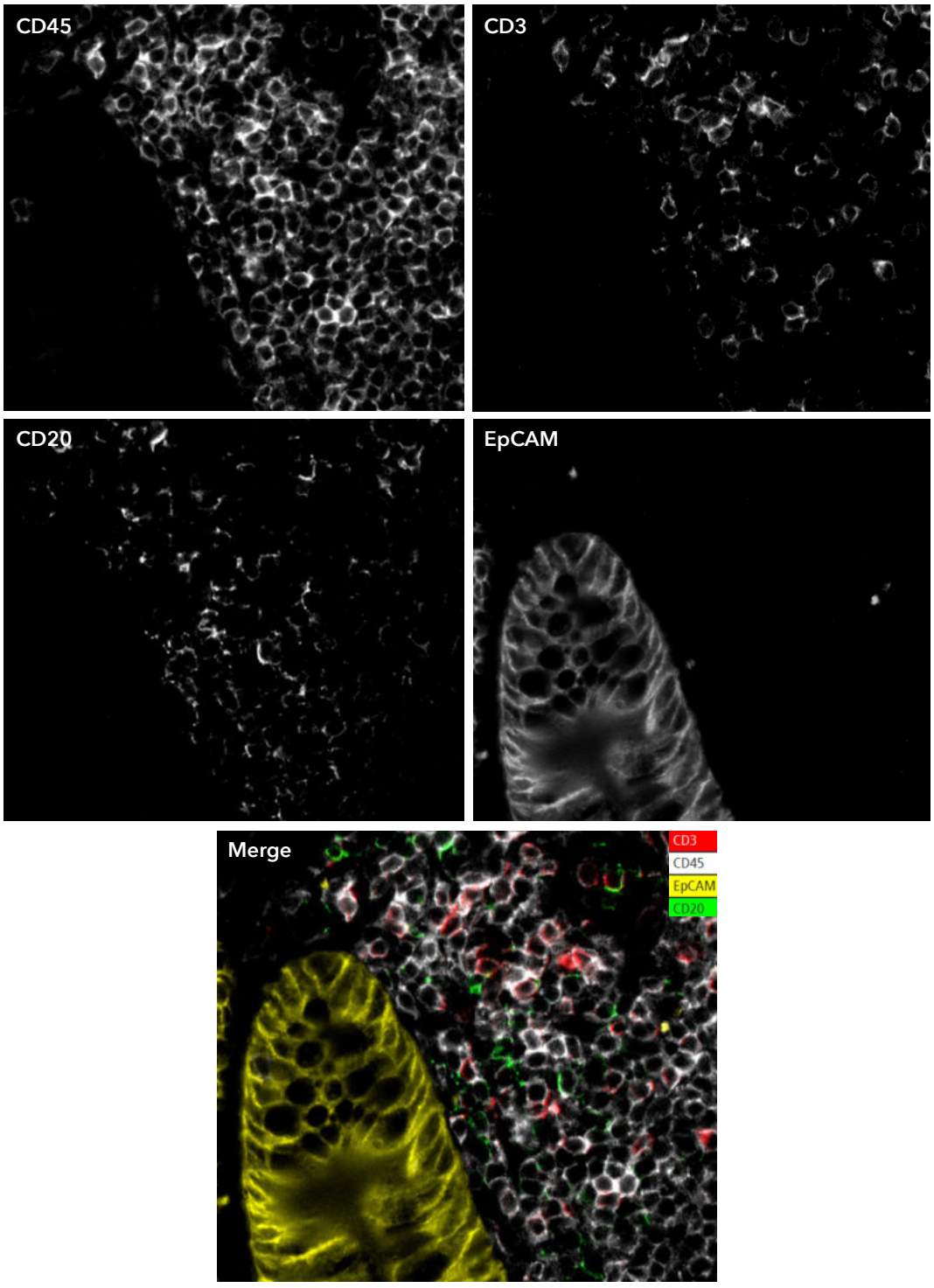


Lung cancer FFPE tissue with select indicated stains shown.

Representative Validation Data (continued)

Specificity

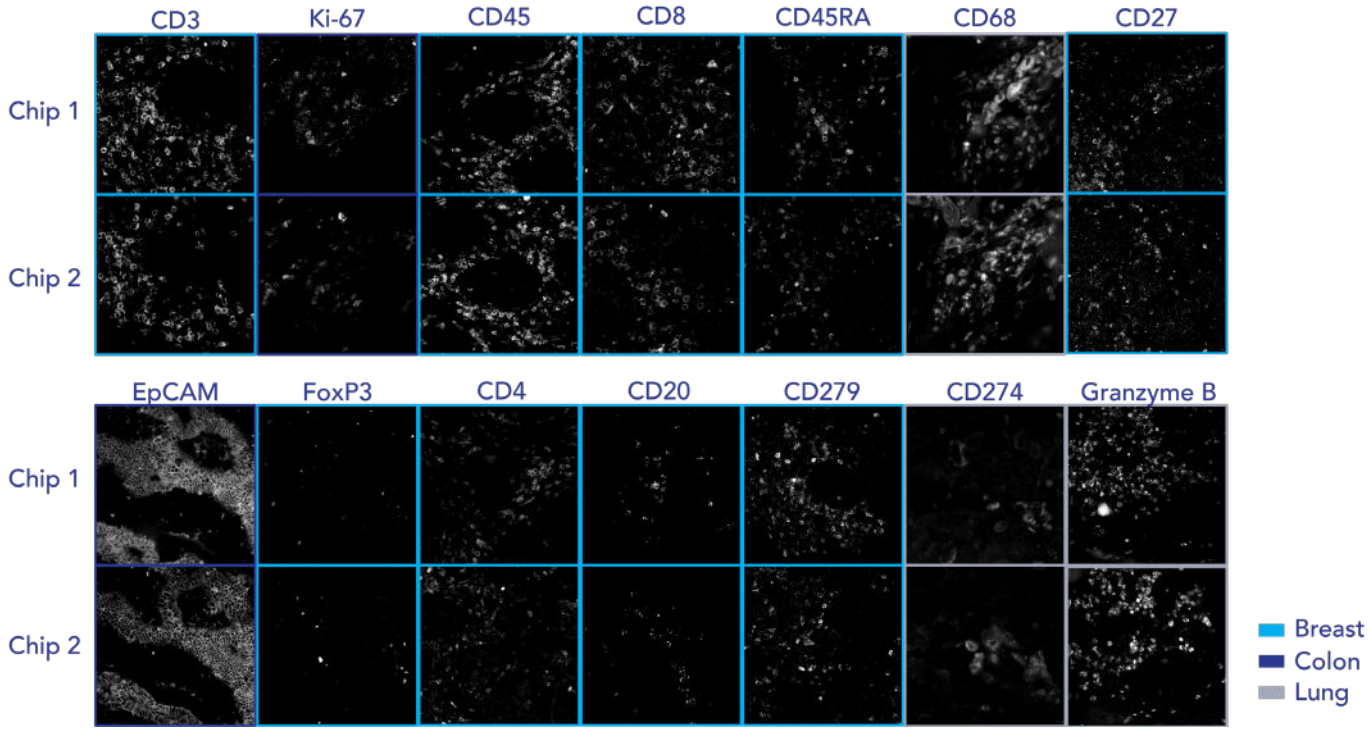
Shown are representative images for specificity assessment of four Spatial Immune Profiling Assay Kit antibodies on a colorectal cancer FFPE sample. CD45 staining overlaps with both CD3 and CD20 as expected for immune cells. CD3 and CD20 antibodies stain non-overlapping cell populations as expected for T cells and B cells, respectively. EpCAM staining is present on structurally distinct mucosal epithelia and does not overlap with immune cell markers.



Representative Validation Data (continued)

Reproducibility

Adjacent FFPE tissue microarray sections containing breast, colon and lung cancer cores were tested with the Spatial Immune Profiling Assay Kit in parallel on a single instrument to demonstrate intra-assay repeatability.



Technical Support

For additional technical support, contact info@canopybiosciences.com

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