VistaPlex™ Lymphoid and Myeloid Assay Kit

CellScape™ Multiplexed Assay Kit for Human PBMC Samples

AbKT-1001-10RXN

Overview

Description

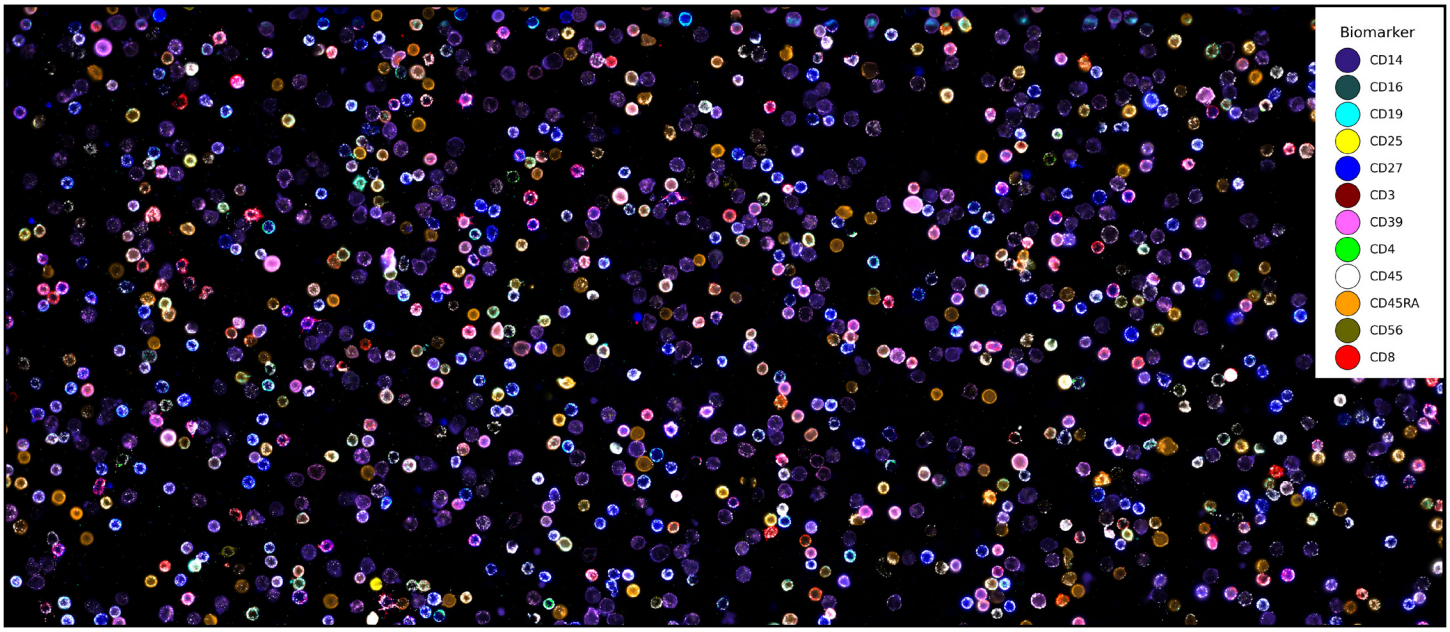
VistaPlex Assay Kits contain ready-to-use, reliable reagents and optimized protocols enabling researchers to obtain quick, robust data with the CellScape platform. Designed to provide a convenient, flexible, and modular analysis of immune cells, the Lymphoid and Myeloid Assay Kit enables relative quantification of 12 phenotypic biomarkers that define T Cell, B Cell, Leukocyte, NK Cell, NKT Cell and Monocyte Cell populations and sub-populations in human PMBC samples.

Each Lymphoid and Myeloid Assay Kit contains 12 pre-validated fluorescent antibodies and buffers for staining 10 samples. This kit was validated on Fresh CPT PBMCs and has also been tested on Cyto-Chex® BCT-stored PBMCs and cryopreserved PBMCs. Multiplex assay kit validation is a multi-stage, iterative process to evaluate antibodies for suitability, specificity and reproducibility.

Report Contents

Data Summary

Sample Type	Suitability	Specificity	Reproducibility
Fresh CPT PBMCs	✓	✓	✓
Cyto-Chex PBMCs	✓	✓	✓
Cryopreserved PBMCs	✓	✓	✓



Human PMBCs were stained and imaged using the Lymphoid and Myeloid Assay Kit. Select biomarkers for phenotyping immune cell populations are displayed.

Product Details

Kit Contents

Description	Volume	Cap Color
Anti-CD3 Antibody	100 µL	Red
Anti-CD25 Antibody	100 µL	
Anti-CD39 Antibody	100 µL	
Anti-CD8 Antibody	100 µL	
Anti-CD4 Antibody	100 µL	Orange
Anti-CD27 Antibody	100 µL	
Anti-CD45RA Antibody	100 µL	
Anti-CD45 Antibody	100 µL	
Anti-CD56 Antibody	100 µL	Yellow
Anti-CD16Antibody	100 µL	
Anti-CD14 Antibody	100 µL	
Anti-CD19 Antibody	100 µL	
Antibody Diluent	50 mL	N/A

Storage

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

Shelf Life

9 months from date received.

Fixation Conditions

Fixation is performed by incubating samples in CellScape Fixation Buffer for 45 minutes at 4 °C.

System Compatibility

The Lymphoid and Myeloid Assay Kit has been optimized for use with the CellScape platform. CellScape supports image exports in OME-tiff and png formats for use in any analysis software.

Intended Use

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

Staining Protocol

Panel Set Up

The staining protocol for the Lymphoid and Myeloid Assay Kit is accomplished in three cycles. A single antibody working stock is created for each cycle, following the dilution instructions in the table below. 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.

The Lymphoid and Myeloid Assay Kit has been optimized to maximize throughput by minimizing the number of cycles required for analysis. One open imaging channel for filter set FS395 in each cycle allows for easy customization. Additional cycles may be added for further customization of your high-plex assay.

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	CD3	FSPerCP	10 µL	260 µL	5 min
	CD25	FS560	10 µL		
	CD39	FS488	10 µL		
	CD8	FS421	10 µL		
2	CD4	FSPerCP	10 µL	260 µL	5 min
	CD27	FS560	10 µL		
	CD45RA	FS488	10 µL		
	CD45	FS421	10 µL		
3	CD56	FSPerCP	10 µL	260 µL	5 min
	CD16	FS560	10 µL		
	CD14	FS488	10 µL		
	CD19	FS421	10 µL		

Example Gating Strategy

Cell Population	Parent Gate	Gating Strategy
Leukocytes	All	CD45+
T cells	Leukocytes	CD45+ CD3+
T cytotoxic cells	T cells	CD45+ CD3+ CD4- CD8+
T helper cells	T cells	CD45+ CD3+ CD4+ CD8-
T regulatory cells	T helper cells	CD45+ CD3+ CD4+ CD8- CD25+ CD39+
Naive CD4+ cells	T helper cells	CD45+ CD3+ CD4+ CD8- CD27+ CD45RA+
Central memory CD4+ cells	T helper cells	CD45+ CD3+ CD4+ CD8- CD27+ CD45RA-
Effector CD4+ cells	T helper cells	CD45+ CD3+ CD4+ CD8- CD27- CD45RA+
Effector memory CD4+ cells	T helper cells	CD45+ CD3+ CD4+ CD8- CD27- CD45RA-
Naive CD8+ cells	T cytotoxic cells	CD45+ CD3+ CD4- CD8+ CD27+ CD45RA+
Central memory CD8+ cells	T cytotoxic cells	CD45+ CD3+ CD4- CD8+ CD27+ CD45RA-
Effector CD8+ cells	T cytotoxic cells	CD45+ CD3+ CD4- CD8+ CD27- CD45RA+
Effector memory CD8+ cells	T cytotoxic cells	CD45+ CD3+ CD4- CD8+ CD27- CD45RA-
NK cells	Leukocytes	CD45+ CD3- CD56+
NKT cells	Leukocytes	CD45+ CD3+ CD56+
B cells	Leukocytes	CD45+ CD3- CD19+
Memory B cells	B cells	CD45+ CD3- CD19+ CD27+
Naive B cells	B cells	CD45+ CD3- CD19+ CD27-
Classical monocytes	Leukocytes	CD45+ CD3- CD14+ CD16-
Non-classical monocytes	Leukocytes	CD45+ CD3- CD14+ CD16+

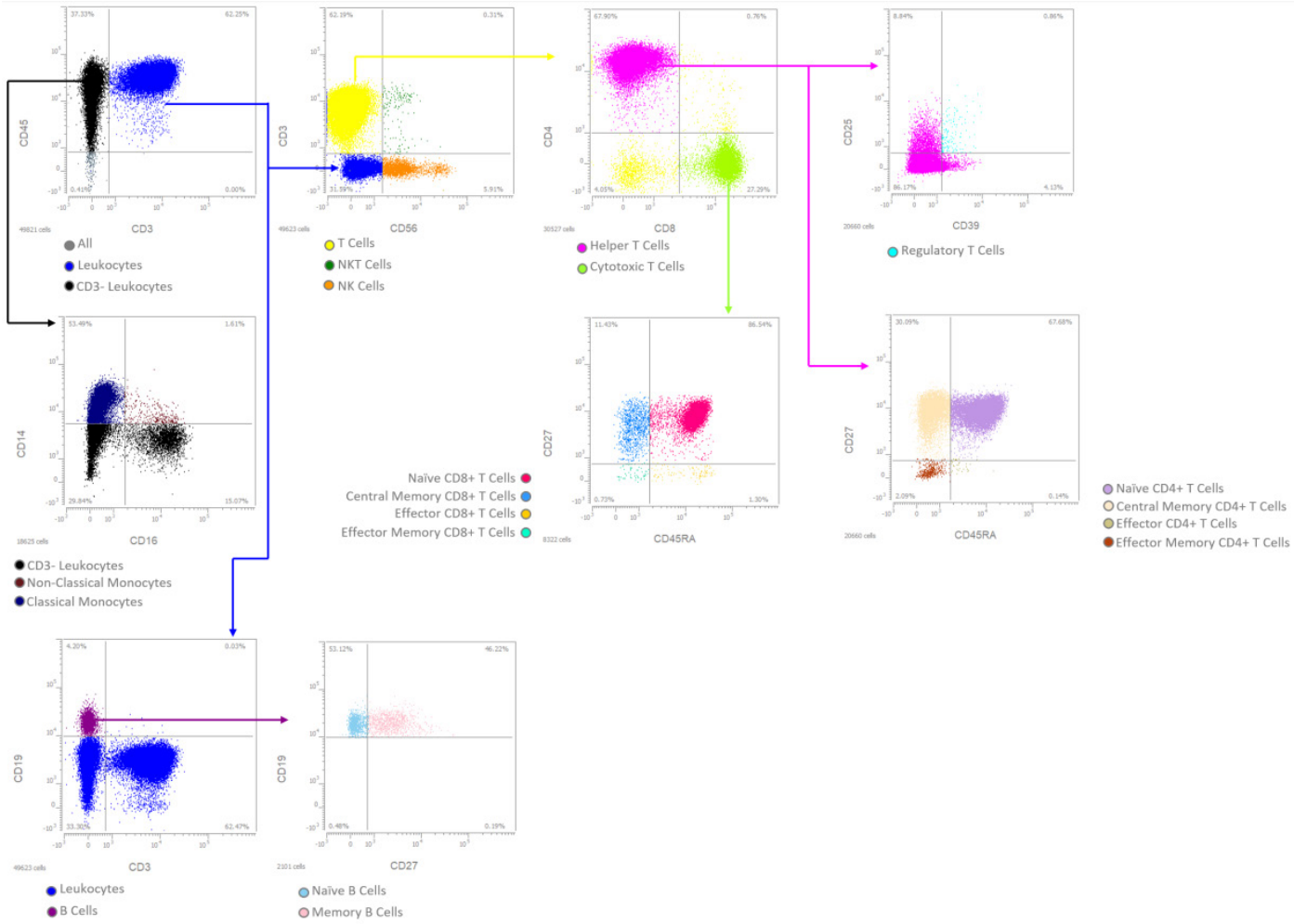
Gating Details

The Lymphoid and Myeloid Assay Kit enables spatial phenotyping of key immune populations including those listed in the table above. Additional phenotypes can be identified for different degrees 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 PBMC samples using the Lymphoid and Myeloid Assay Kit.

Example Gating Strategy (continued)



Assay Validation Process

Antibodies in the Lymphoid and Myeloid Assay Kit have been fully validated for precise and consistent performance in human PBMC suspensions.

Specificity

All assay kit antibodies undergo rigorous testing to ensure antibodies bind their intended targets and do not demonstrate off-target effects. The specificity of each antibody is assessed with appropriate counterstains to ensure that antibodies stain their intended cell types and localize to the expected subcellular regions. 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 Lymphoid and Myeloid Assay Kit was tested on technical replicates from three different donors. Fresh PMBC samples were used to confirm intra-assay and inter-assay reproducibility. To ensure high quality data can be obtained on a variety of sample types, the assay kit was successfully tested on fresh, cryopreserved, and Cyto-Chex BCT-collected samples. 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.

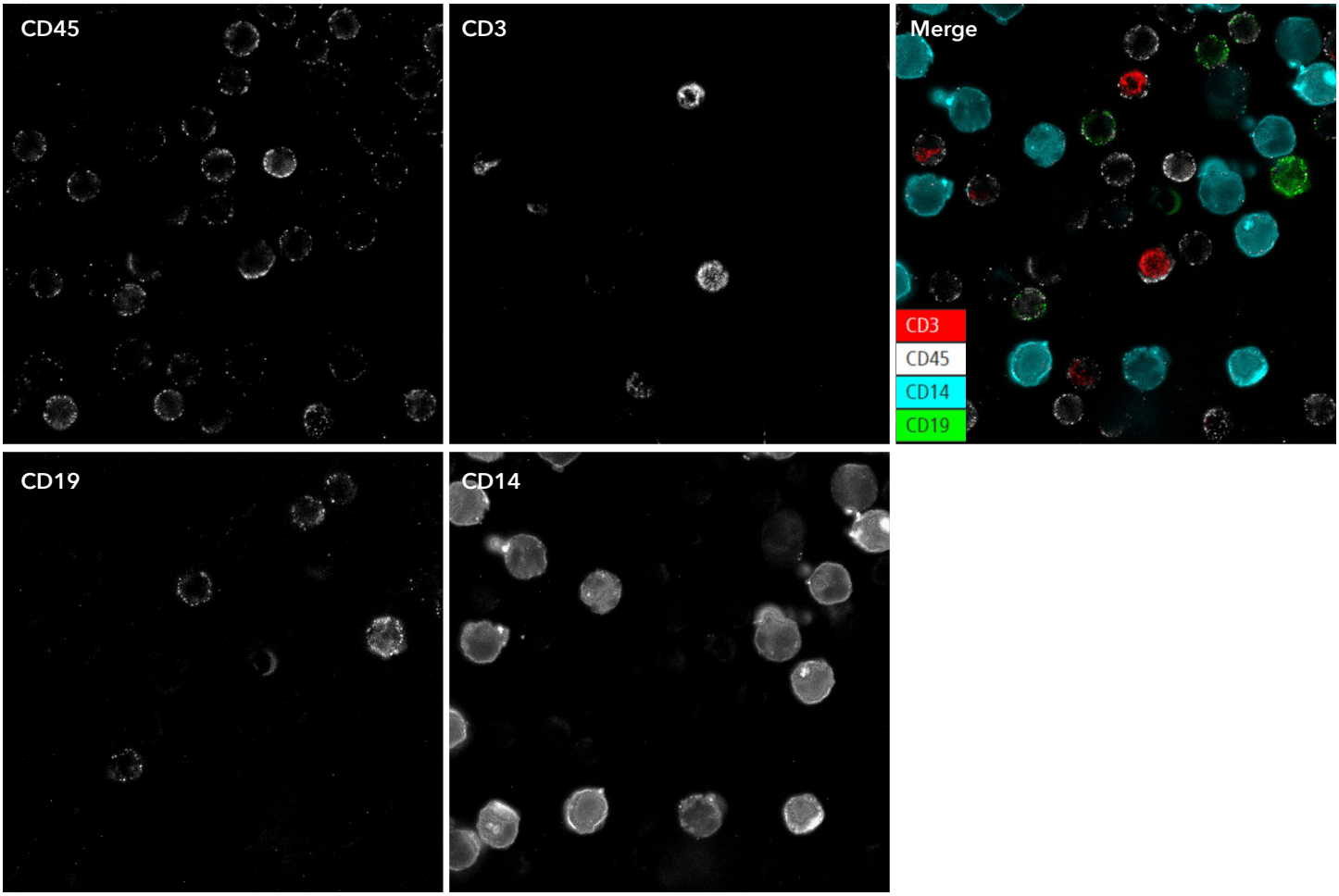
Individual Antibody Validation Results

Marker	Visible Signal	Subcellular Localization	Specific Localized Signal	Review by 2 Analysts
CD3	Pass	Surface	Pass	Pass
CD25	Pass	Surface	Pass	Pass
CD39	Pass	Surface	Pass	Pass
CD8	Pass	Surface	Pass	Pass
CD4	Pass	Surface	Pass	Pass
CD27	Pass	Surface	Pass	Pass
CD45RA	Pass	Surface	Pass	Pass
CD45	Pass	Surface	Pass	Pass
CD56	Pass	Surface	Pass	Pass
CD16	Pass	Surface	Pass	Pass
CD14	Pass	Surface	Pass	Pass
CD19	Pass	Surface	Pass	Pass

Representative Validation Data

Specificity

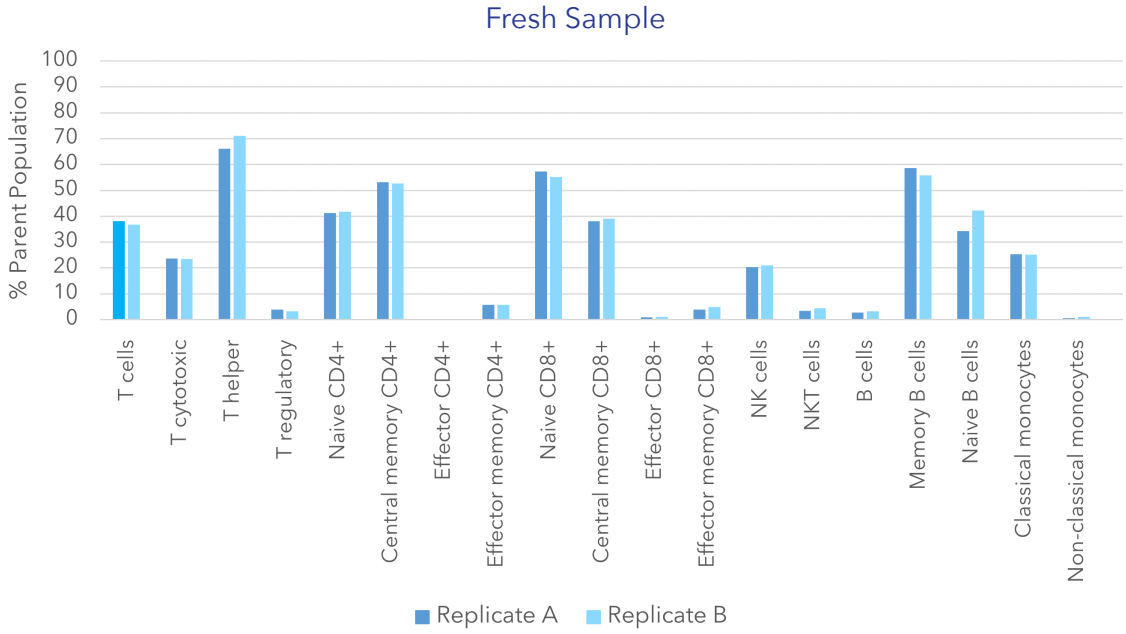
Shown are representative images for specificity assessment of four Lymphoid and Myeloid Assay Kit antibodies. CD45 staining overlaps with both CD3 and CD19 as expected for immune cells. CD3 and CD19 antibodies stain non-overlapping cell populations as expected for T cells and B cells, respectively. CD14 staining is present on morphologically distinct myeloid cells and does not overlap with lymphocyte markers.

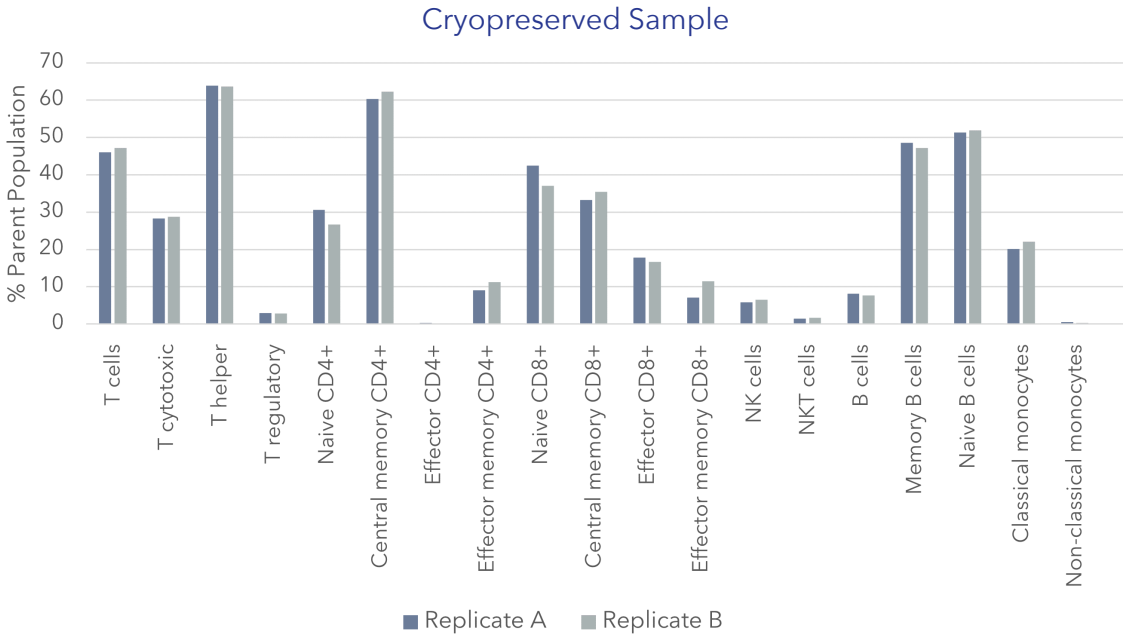


Representative Validation Data (continued)

Reproducibility

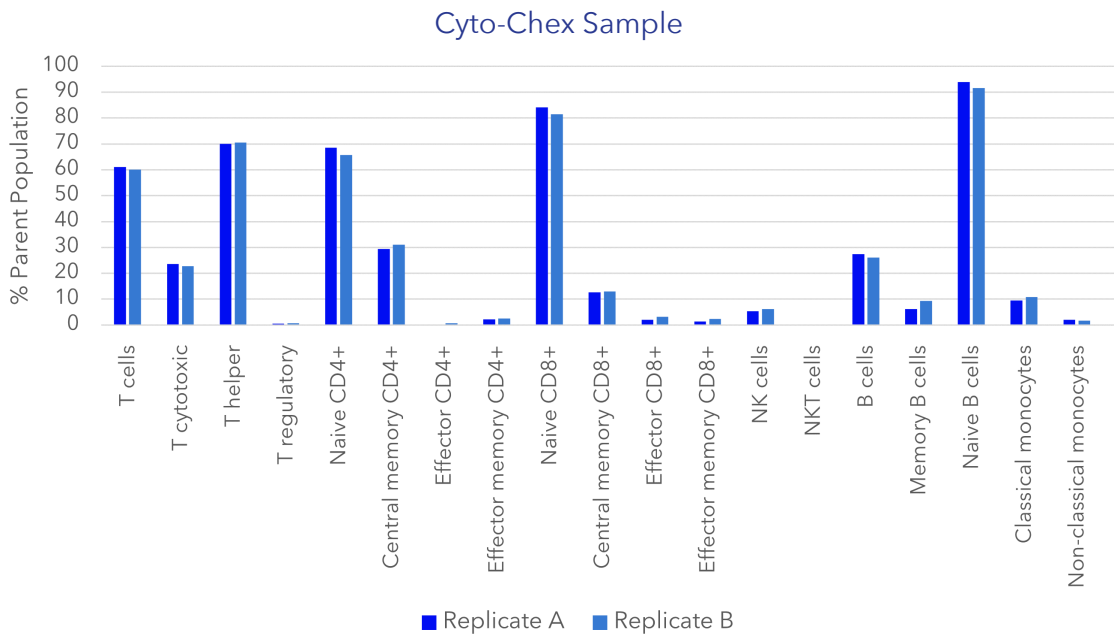
The Lymphoid and Myeloid Assay Kit was demonstrated to produce reproducible and consistent results across samples storage types by assaying technical duplicates from different donors with three different sample storage methods. Leukocyte populations were quantified as percentage of parent population using the gating strategy shown on page 4. Shown below are quantitative cell phenotyping results, with similar colors represent technical replicates from the same donor.





Representative Validation Data (continued)

Reproducibility (continued)



Technical Support

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