

## PRODUCT DESCRIPTION

T cells and B cells are critical mediators of adaptive immunity. This panel contains 3 conjugated antibodies that can be used for the single step staining of T cells and B cells.

## INTENDED USE

This multicolor flow cytometry panel was validated on human peripheral blood mononuclear cells (PBMCs).

## MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C. Do not use past expiration date.

MARKER	CLONE	FLUOROCROME	SIZE	RECOMMENDED CONCENTRATION
CD3	UCHT1	Alexa Fluor® 405	100 tests	5 µL/10 <sup>6</sup> cells
CD19	4G7-2E3	PE	100 tests	10 µL/10 <sup>6</sup> cells
CD45	2D1	Alexa Fluor® 647	100 µg	0.25-1 µg/10 <sup>6</sup> cells

*Note: Recommended concentrations are given as reference point for antibody titration. Optimal concentrations should be determined by each laboratory for their experimental conditions.*

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

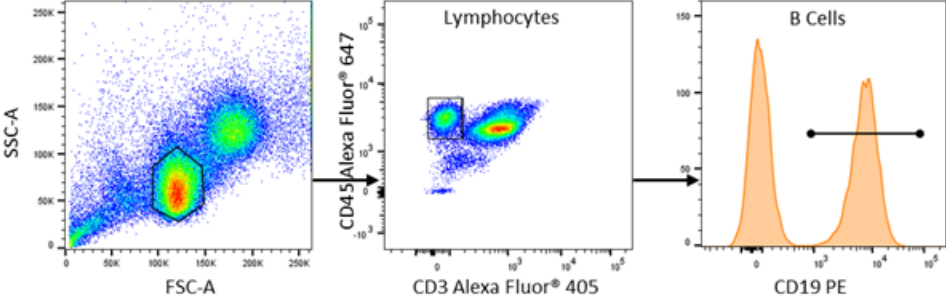
## OTHER SUPPLIES REQUIRED

- PBS
- Flow Cytometry Staining Buffer ([R&D Systems®, Catalog # FC001](#))
- Fc-block (blocking IgG)
- Isotype Control Antibodies (optional)
- 5 mL Flow cytometry tubes

## PROTOCOL

1. Wash human PBMCs (1 x 10<sup>6</sup> cells per sample) with 2 mL of Staining Buffer (1X) ([R&D Systems® Catalog # FC001](#)) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes. Decant/aspirate supernatant.
2. Fc-block cells with blocking IgG (1 µg IgG/10<sup>6</sup> cells) for 10 minutes at room temperature.
3. Add previously titrated amount of each primary conjugated antibody. Vortex tubes.
4. (Optional) To a separate tube, add 5 µL of each of the isotype control antibodies. Vortex tubes.
5. Incubate the mixtures for 30-45 minutes at room temperature **in the dark**.
6. Wash with 2 mL of Staining Buffer (1X), by spinning at 300 x g for 5 minutes at end of incubation. Decant/aspirate supernatant.
7. Resuspend the cells in 0.2 - 0.5 mL Staining Buffer (1X) and acquire on a Flow Cytometer.

**DATA EXAMPLES**



**Multicolor Flow Cytometry Panel to identify Human B Cells.** PBMCs were stained with Anti-Human CD45 Alexa Fluor<sup>®</sup> 647, CD3 Alexa Fluor 405, and CD19 PE. All antibodies are validated for flow cytometry.