

Summary

Antigen	anti-CD20 antibody (Membrane-Spanning 4-Domains, Subfamily A, Member 1) (APC)
Catalog number	ABIN2104927
Supplier	Sino Biologicals
Supplier catalog number	11007-R001A
Lot number	HG08OC1306
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29848
Positive Control	peripheral blood mononuclear cells
Negative Control	peripheral blood mononuclear cells
Notes	None



Full Methods

Primary Antibody

- Antigen: human CD20 (CD20)
- Catalog number: ABIN2104927
- Supplier: Sino Biologicals
- Supplier catalog number: 11007-R001A
- Lot number: HG08OC1306
- Dilution: 10 μ l in 100 μ L 1X PBS containing 0.5% BSA

Controls

- Positive control: peripheral blood mononuclear cells stained with anti-CD20
- Negative control: peripheral blood mononuclear cells that are CD20 negative and unstained cells
- Isotype Control: None
- Secondary Only control: None

Protocol

10mls of blood (collected in EDTA) was diluted to 20mls with PBS + 0.5% BSA

10mls of diluted blood was overlaid onto 5mls of ficoll.

Spin for 20 minutes at 1500 rpm with break off.

The buffy coat layer was collected and wash with PBS/BSA.

Residual red blood cells were lysed by resuspending the cells in 1ml ammonium chloride for 5-10 minutes at room temperature.

The cells were washed with PBS/BSA, counted and resuspended at 10×10^6 cells/ml.

100 μ l of cells (1×10^6) were added to their respective tubes.

Add 10 μ l of anti-CD20-APC (Sino Biological) to the appropriate tubes and incubate for 20 minutes on ice.

Add 1ml PBS/BSA. Spin the cells down and resuspend in 0.5ml PBS/BSA

Analyze

Experimental Notes

Figures

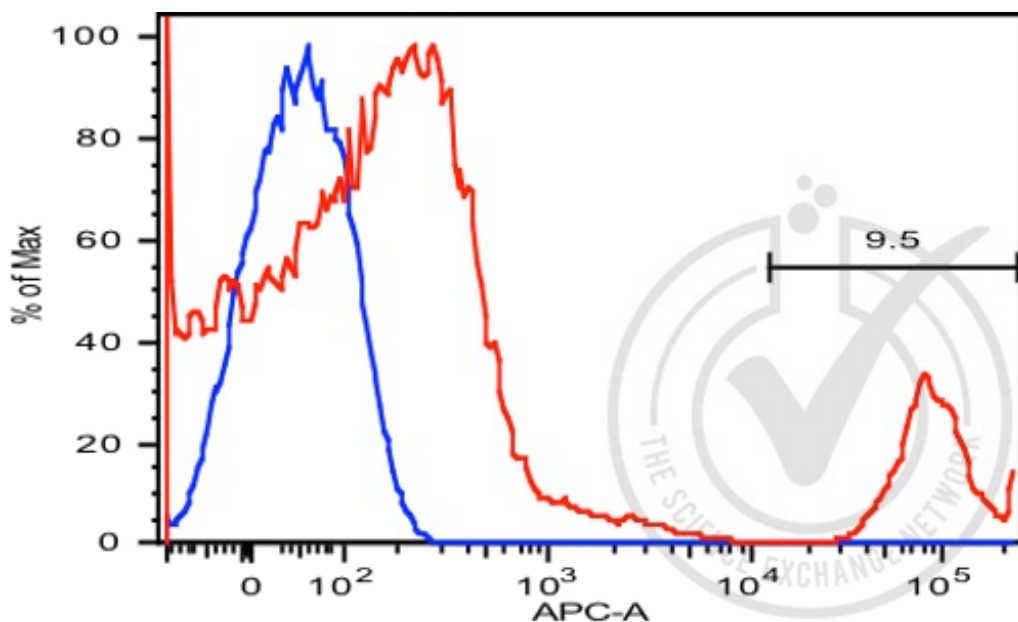


Figure 1: Histogram of peripheral blood lymphocytes stained with anti-CD20-APC (red), unstained cells are shown in blue. Percentage of CD20+ cells is 9.5%.

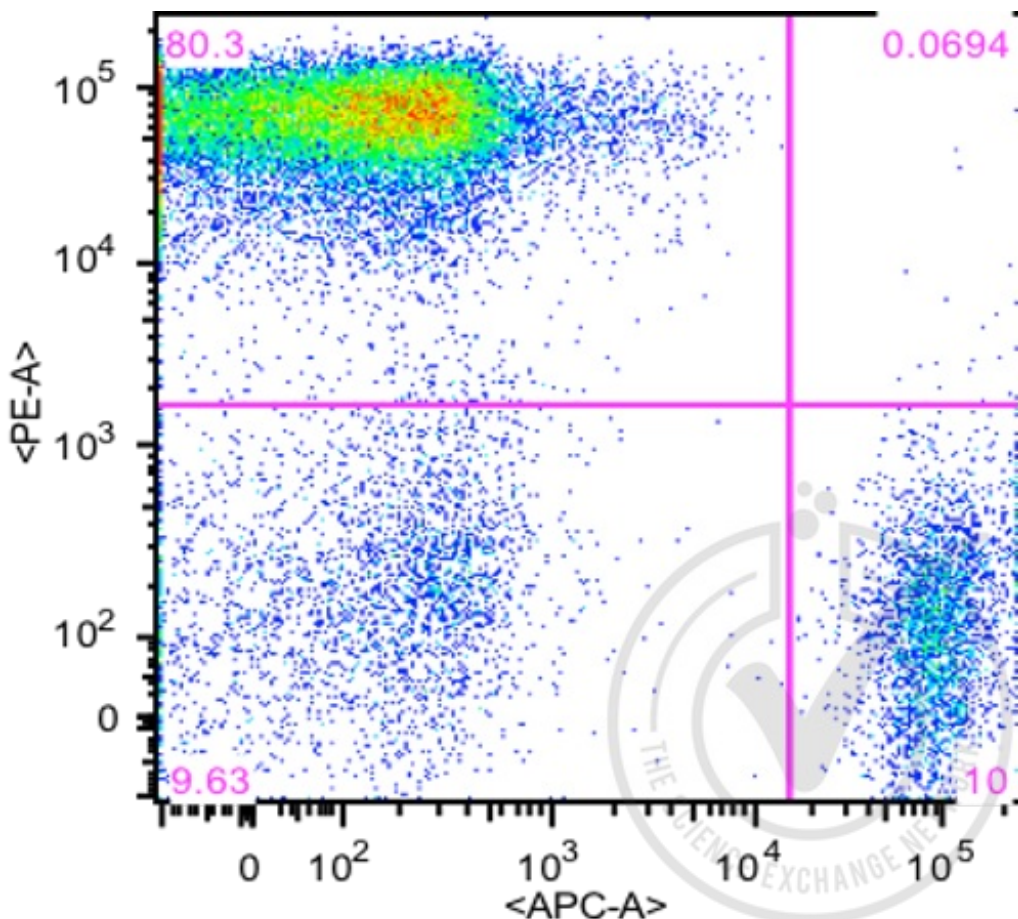


Figure 2: Dot plot of peripheral blood lymphocytes stained with anti-CD20-APC (x-axis) and anti-CD3-PE (y-axis).