

Summary

Antigen	anti-CD4 antibody (CD4 Molecule) (FITC)
Catalog number	ABIN2104933
Supplier	Sino Biologicals
Supplier catalog number	10400-MM08-F
Lot number	HG08FE1721
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29847
Positive Control	peripheral blood mononuclear cells
Negative Control	peripheral blood mononuclear cells
Notes	This antibody performed well and is comparable to anti-CD4-FITC from BD Pharmingen (see data below)



Full Methods

Primary Antibody

- Antigen: human CD4 (CD4)
- Catalog number: ABIN2104933
- Supplier: Sino Biologicals
- Supplier catalog number: 10400-MM08-F
- Lot number: HG08FE1721
- Dilution: 1 μ L in 100 μ L 1X PBS containing 0.5% BSA

Controls

- Positive control: peripheral blood mononuclear cells stained with anti-CD4
- Negative control: peripheral blood mononuclear cells that are CD4 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.

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

Add 5 μ L of anti-CD4-FITC (Sino Biological) or 20 μ L of anti-CD4-FITC (BD Pharmingen) 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

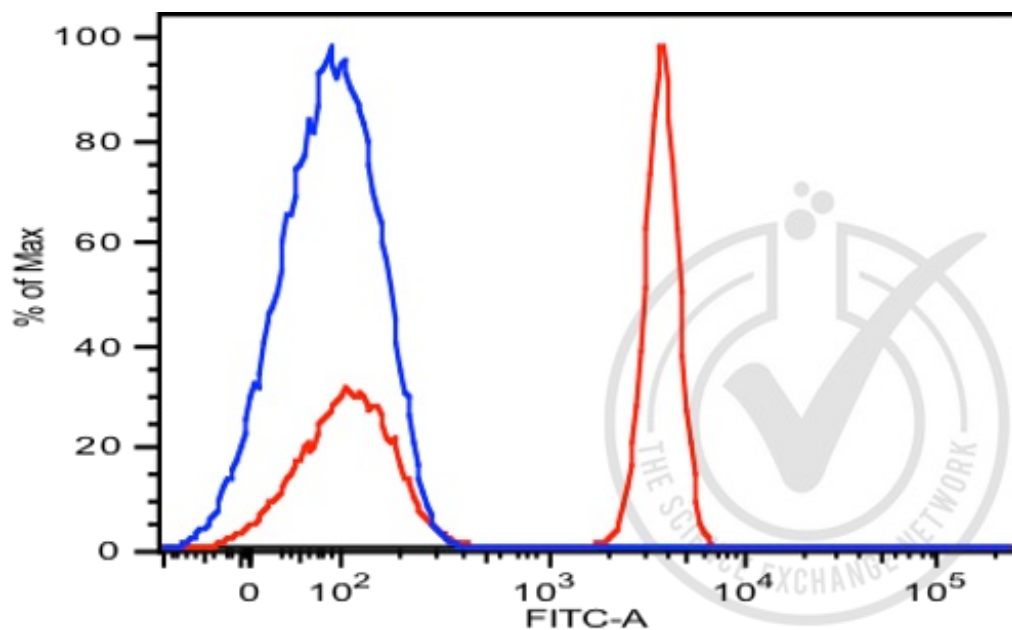


Figure 1: Histogram of peripheral blood lymphocytes stained with anti-CD4-FITC (red), unstained cells are shown in blue. Percentage of CD4+ cells is 52.7%, median fluorescence intensity is 3797.

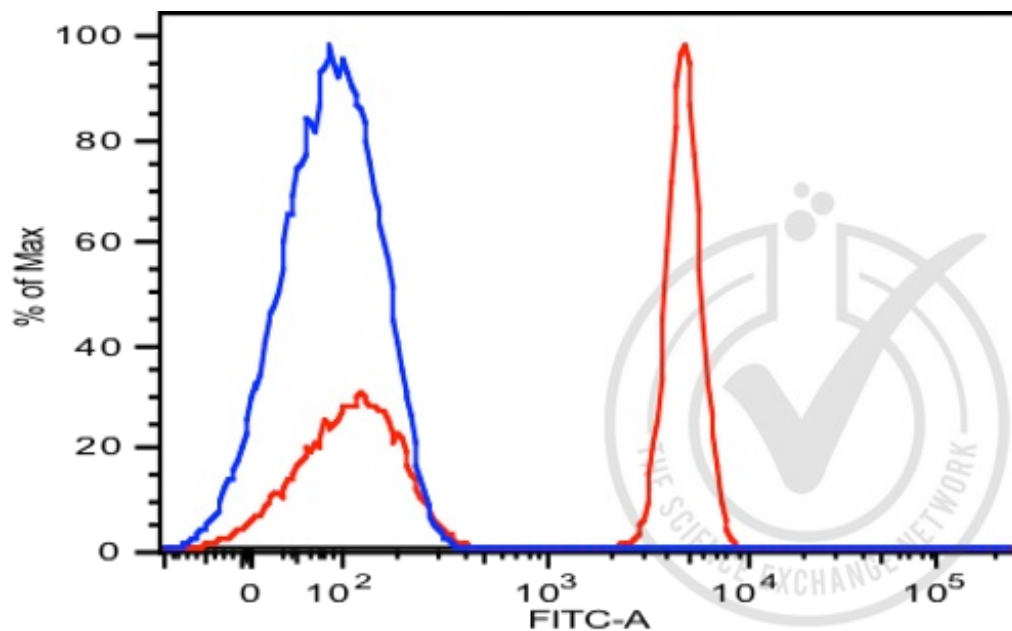


Figure 2: Histogram of peripheral blood lymphocytes stained with anti-CD4-FITC from BD Pharmingen (red), unstained cells are shown in blue. Percentage of CD4+ cells is 52.5%, median fluorescence intensity is 4901.

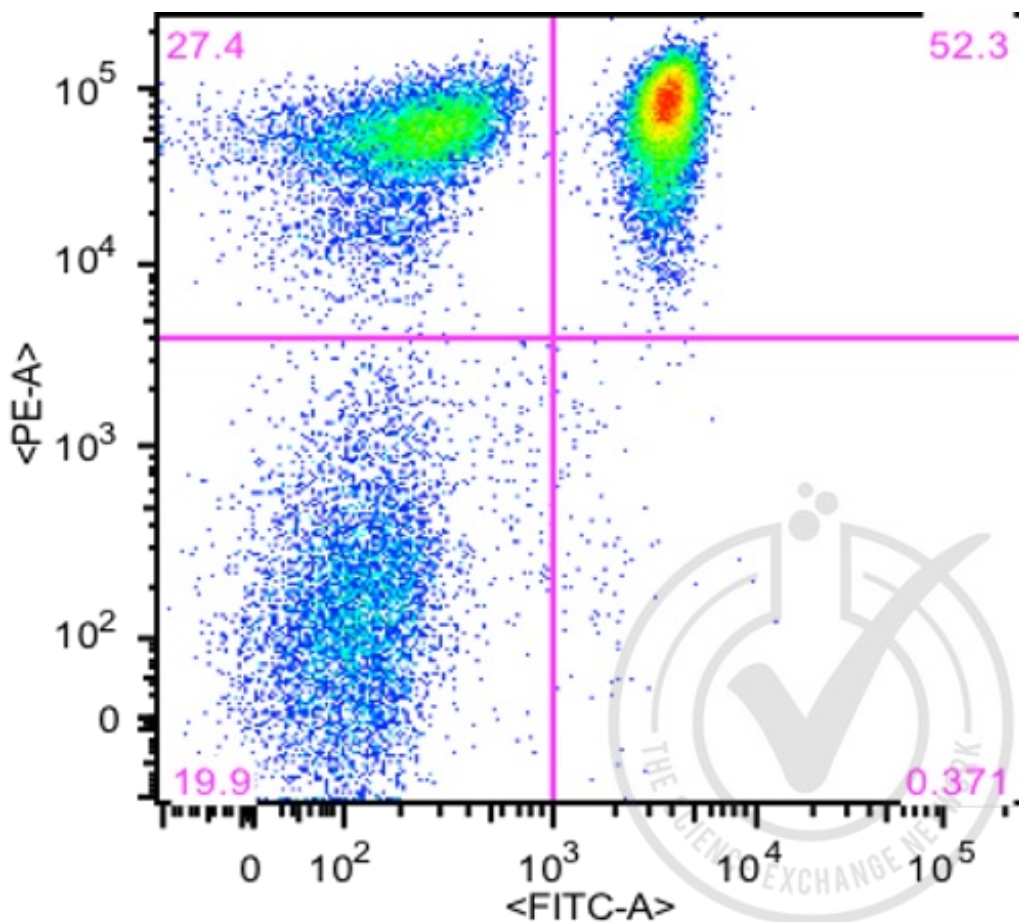


Figure 3: Dot plot of peripheral blood lymphocytes stained with anti-CD4-FITC (x-axis) and anti-CD3-PE (y-axis).