Validation Report #029617

Validation Date: 02/22/14

Summary

Antigen	Prostate Specific Antigen (PSA)
Catalog number	ABIN1543584
Supplier	Yashraj Biotechnology
Supplier catalog number	fpsamab-37/1
Lot number	FAPS-A5D5-0003
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29617
Positive Control	LNCaP cells
Negative Control	• DU145 cells • Jurkat cells
Notes	Strong signal above isotype signal was observed in the positive control sample, while staining of similar level to isotype control was observed in both negative control samples.

Full Methods

Primary Antibody
- Antibody: Prostate Specific Antigen (PSA)
- Catalog number: ABIN1543584
- Supplier: Yashraj Biotechnology
- Supplier catalog number: fpsamab-37/1
- Lot number: FAPS-A5D5-0003

Isotype Control Antibody
- Antibody: mouse IgG1
- Supplier: BD Biosciences
- Catalog number: 557273

Secondary Antibody
- Antibody: F(ab')2 goat anti-mouse IgG-Alexa 647
- Supplier: Jackson Immunoresearch
- Catalog number: 115-606-146

Controls
- Positive control: LNCaP prostate tumor cell line
- Negative control: DU145 prostate tumor cell line, Jurkat T cell leukemia cell line
- Isotype control: cells treated with mouse IgG1 instead of the primary antibody to confirm that primary antibody binding is specific.

Protocol
- Positive and negative control cells were washed once with phosphate-buffered saline (PBS) and harvested with 0.05% trypsin / 0.025% EDTA.
- Detached cells were washed and resuspended in 0.5 mL 1X PBS containing 0.5% BSA.
- An equivalent amount of pre-warmed 4% paraformaldehyde was added and the cells were incubated for 10 min at 37°C.
- Cells were washed in 1X PBS containing 0.5% BSA and resuspended in 1 mL of ice-cold 90% methanol for 30 min on ice.
- Cells were spun out of the methanol and washed twice by resuspending in 1X PBS containing 0.5% BSA and 0.1% Tween 20
- Cells were resuspended in 100 µL 1X PBS containing 0.5% BSA.
- 1 µg of anti-PSA or mouse IgG1 isotype control were added to the cells and incubated for 30 min at room temperature
- Cells were washed twice with PBS/BSA/Tween 20
- Cells were resuspended in 100 µL 1X PBS containing 0.5% BSA
- Secondary antibody was added at a final dilution of 1:500 and incubated for 30 min at room temperature
- Cells were washed twice with PBS/BSA/Tween 20
- Cells were resuspended in 0.3 mL PBS/BSA
- Cells were analyzed on a FACSAria III (BD Biosciences) using a red laser (640 nm excitation / 660 nm emission).

Experimental Notes

None

Figures

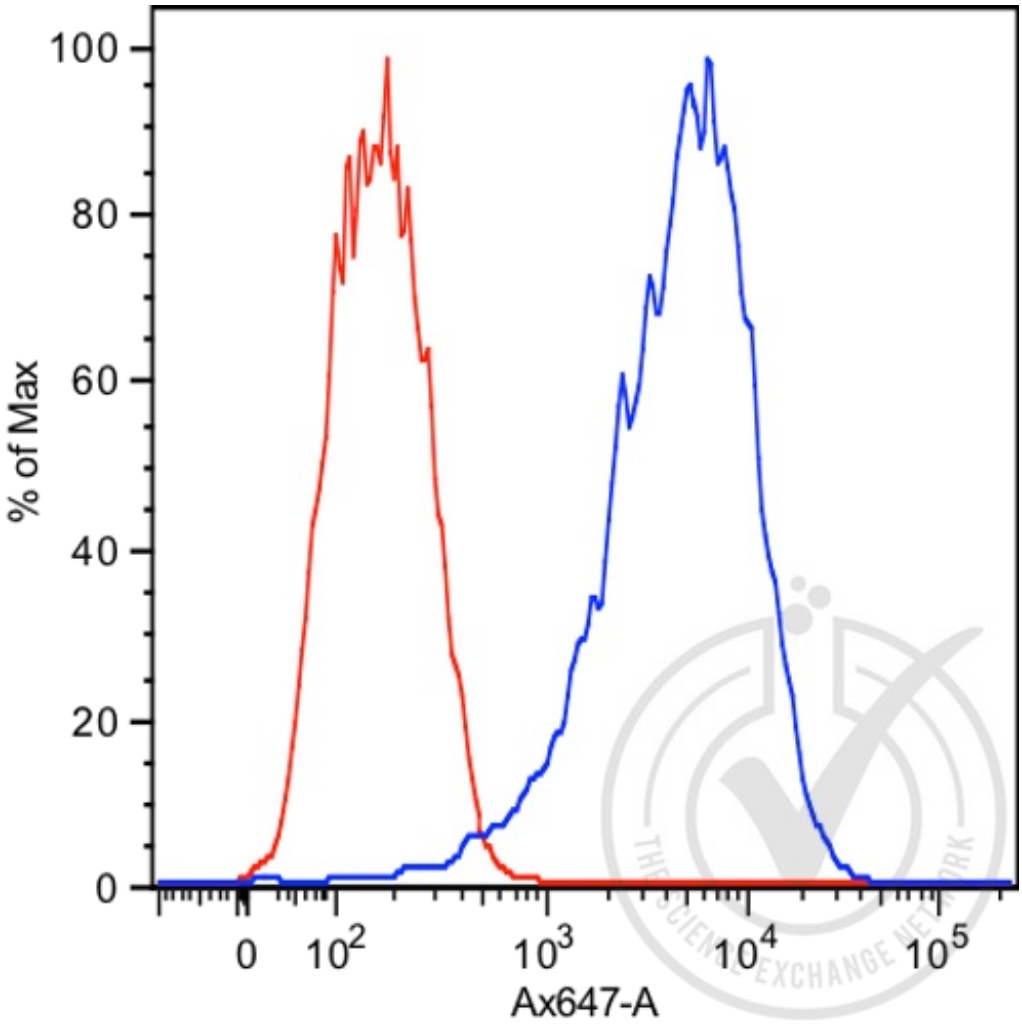

Figure 1: LNCaP cells stained with anti-PSA (blue) or with isotype control (red).

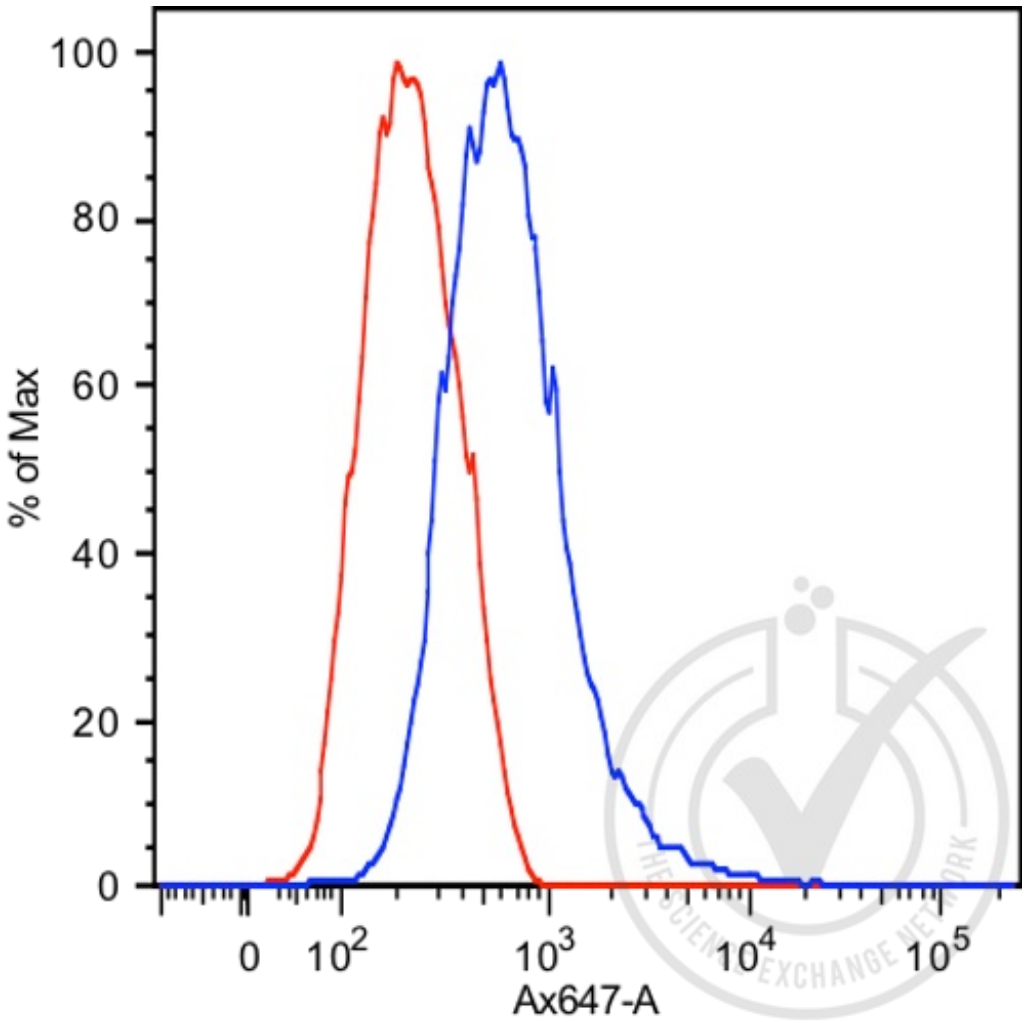

Figure 2: DU145 cells stained with anti-PSA (blue) or with isotype control (red).

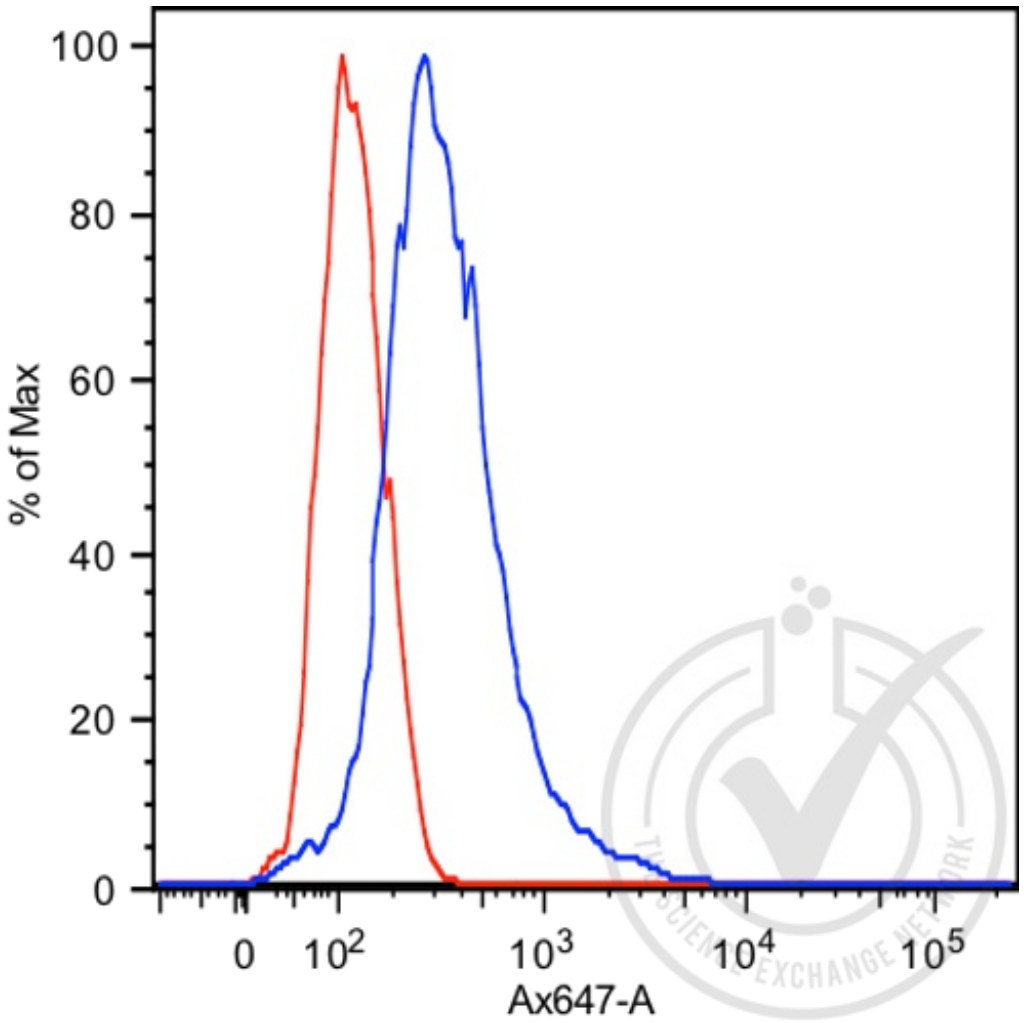

Figure 3: Jurkat cells stained with anti-PSA (blue) or with isotype control (red).