Validation Report #029769

Validation Date: 07/22/14

Summary

Antigen	V-Akt Murine Thymoma Viral Oncogene Homolog 1 (AKT1)
Catalog number	ABIN725195
Supplier	Bioss
Supplier catalog number	bs-0115R
Lot number	131113
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29769
Positive Control	MCF-7 cells - high expression
Positive Control	MDA-MB-468 cells - lower expression
Notes	A strong specific signal is observed in both of the positive control cells stained with anti-AKT1 plus secondary antibody compared with isotype, secondary only and unstained cells.

Full Methods

Primary Antibody
- Antigen: V-Akt Murine Thymoma Viral Oncogene Homolog 1 (AKT1)
- Catalog number: ABIN725195
- Supplier: Bioss
- Supplier catalog number: bs-0115R
- Lot number: 131113
- Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA

Isotype Control Antibody
- Antibody: Rabbit IgG
- Catalog number: 3900S
- Supplier: Cell Signaling Technology
- Lot number: 16
- Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA

Secondary Antibody
- Antibody: Goat anti-rabbit IgG-FITC
- Supplier: Jackson Immunoresearch
- Catalog number: 111-096-144
- Lot number: 115629
- Dilution: 1:200 in 1X PBS containing 0.5% BSA

Controls
- Positive control: MCF-7 cells
- Low positive control: MDA-MB-468 cells
- Isotype control: Both cell lines treated with rabbit IgG instead of the primary antibody to confirm that primary antibody binding is specific.
- Secondary only control: Both cell lines treated with Goat anti-rabbit IgG-FITC to confirm no background signal produced from secondary antibody alone

Protocol
- Control cells were cultured in DMEM + 10% FBS.
- Control cells were washed once with phosphate-buffered saline (PBS) and harvested with a non-enzymatic cell dissociation solution (Cellstripper, Mediatech, Inc).
- 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.
- Cells were resuspended in 100 µL 1X PBS containing 0.5% BSA:
  - unstained cells
  - secondary antibody alone
  - isotype control antibody + secondary antibody
  - primary antibody + secondary antibody
- Cells were incubated for 30 min on ice.
- Labeled cells were washed twice in PBS containing 0.5% BSA.
- Cells were resuspended with 1X PBS containing 0.5% BSA + 10% goat serum and incubated for 15 min at room temperature.
- Goat anti-rabbit IgG-FITC secondary antibody (Jackson Immunoresearch) was added at a 1:200 dilution. The cells were incubated for 30 min in the dark on ice.
- Labeled cells were washed twice in PBS containing 0.5% BSA.

- Cells were analyzed on a FACSAria III (BD Biosciences) using a blue laser (488 nm excitation / 525 nm emission).

Experimental Notes

Nothing to note.

Figures

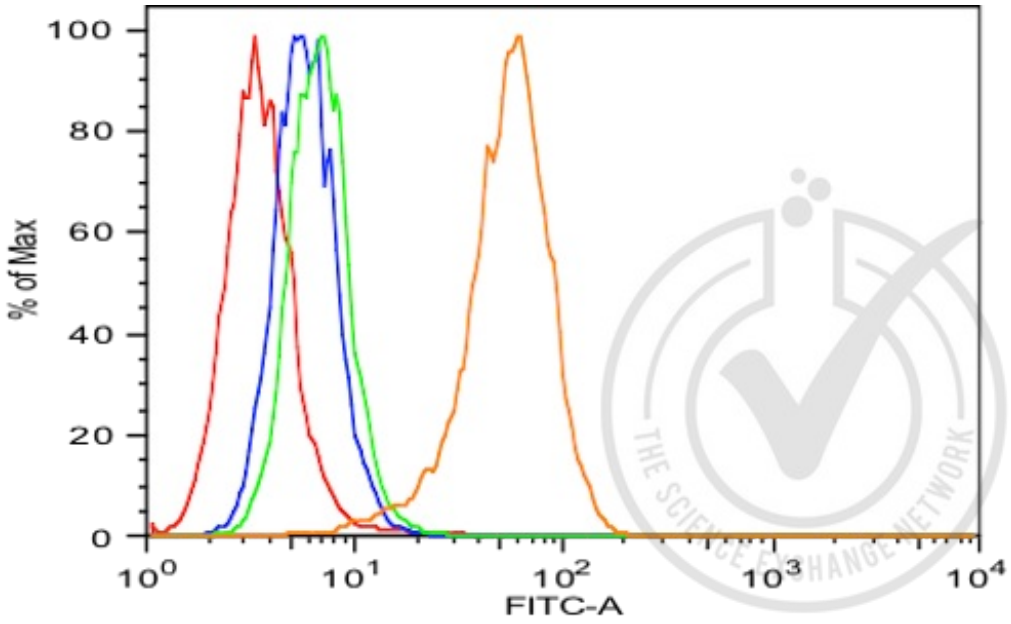

Figure 1: Positive control (high expression) MCF7 cells. The red histogram is unstained cells, the blue histogram is cells stained with secondary antibody alone, the green histogram is cells stained with rabbit IgG isotype control antibody plus secondary antibody and the orange histogram is cells stained with anti-AKT1 plus secondary antibody. The secondary antibody is a goat anti-rabbit IgG-FITC (Jackson Immunoresearch).

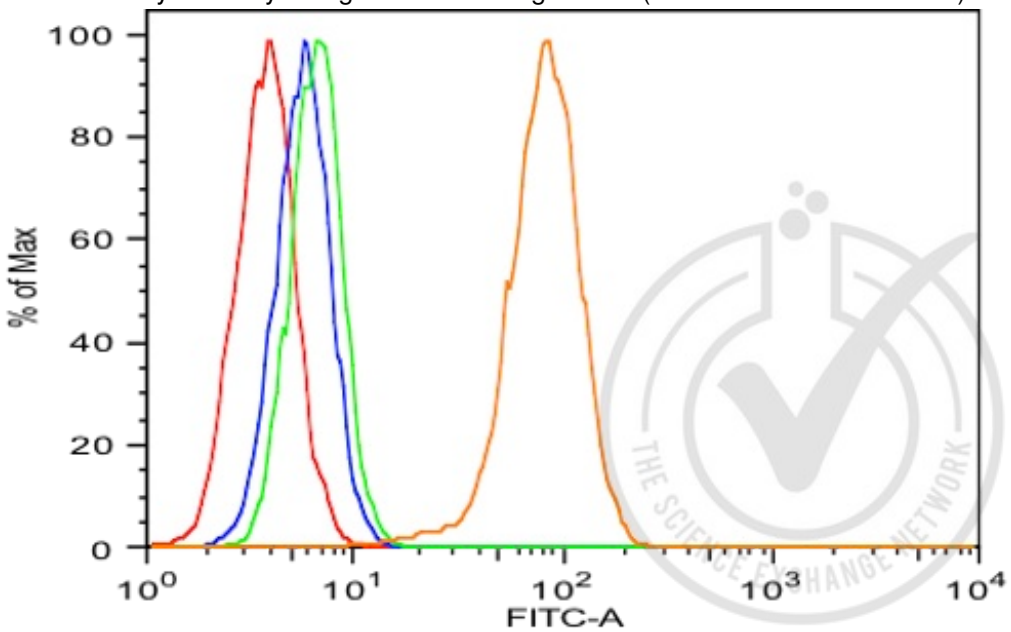

Figure 2: Positive control (lower expression) MDA-MB-468 cells. The red histogram is unstained cells, the blue histogram is cells stained with secondary antibody alone, the green histogram is cells stained with rabbit IgG isotype control antibody plus secondary antibody and the orange histogram is cells stained with anti-AKT1 plus secondary antibody. The secondary antibody is a goat anti-rabbit IgG-FITC (Jackson Immunoresearch).