Validation Report #029768

Validation Date: 07/22/14

Summary

Antigen	Cadherin 1, Type 1, E-Cadherin (Epithelial) (CDH1)
Catalog number	ABIN1387847
Supplier	Bioss
Supplier catalog number	bs-10009R
Lot number	130902
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29768
Positive Control	MCF-7 cells
Negative Control	SH-SY5Y cells
Notes	A weak but specific signal is observed in the positive control MCF7 cells stained with anti-E-Cadherin plus secondary antibody compared with isotype, secondary only and unstained cells. No staining was observed in the negative control SH-SY5Y cells as expected.

Full Methods

Primary Antibody
- Antigen: Cadherin 1, Type 1, E-Cadherin (Epithelial) (CDH1)
- Catalog number: ABIN1387847
- Supplier: Bioss
- Supplier catalog number: bs-10009R
- Lot number: 130902
- 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
- Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA

Secondary Antibody
- Antibody: Goat anti-rabbit IgG-Alexa 647
- Supplier: Jackson Immunoresearch
- Catalog number: 712-606-150
- Dilution: 1:500 in 1X PBS containing 0.5% BSA

Controls
- Positive control: MCF-7 cells
- Negative control: SH-SY5Y 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-Alexa 647 to confirm no background signal produced from secondary antibody alone

Protocol
- Positive and negative control cells were cultured in DMEM + 10% FBS.
- Positive and negative 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 twice and 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-Alexa 647 secondary antibody (Jackson Immunoresearch) was added at a 1:500 dilution. The cells were incubated for 30 min in the dark on ice.
- Labeled cells were washed twice in PBS containing 0.5% BSA.
- Propidium Iodide (PI) was added to discern live cells from dead cells.
- Cells were analyzed on a FACSAria III (BD Biosciences) using a red laser (640 nm excitation / 660 nm emission).

Experimental Notes
- The data displayed is gated on PI negative cells.

Figures

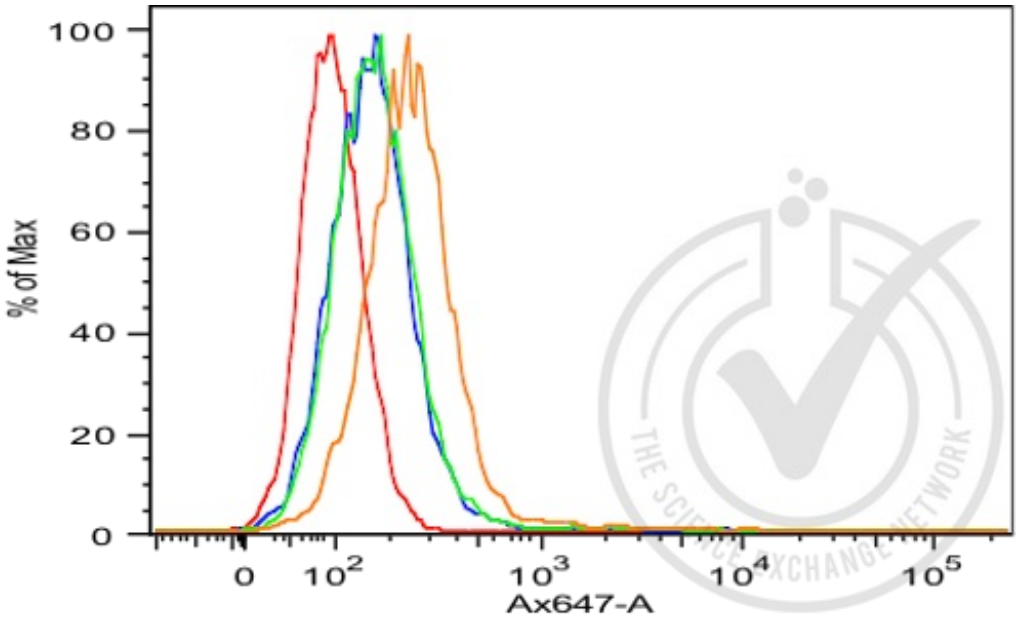

Figure 1: Positive control MCF7 cells. The red histogram is unstained cells, the blue histogram is cells stained with secondary antibody alone, the green histogram is cells stained with rabbit IgG isotype control antibody plus secondary antibody and the orange histogram is cells stained with anti-E-Cadherin plus secondary antibody. The secondary antibody is a goat anti-rabbit IgG-Alexa 647 (Jackson Immunoresearch).

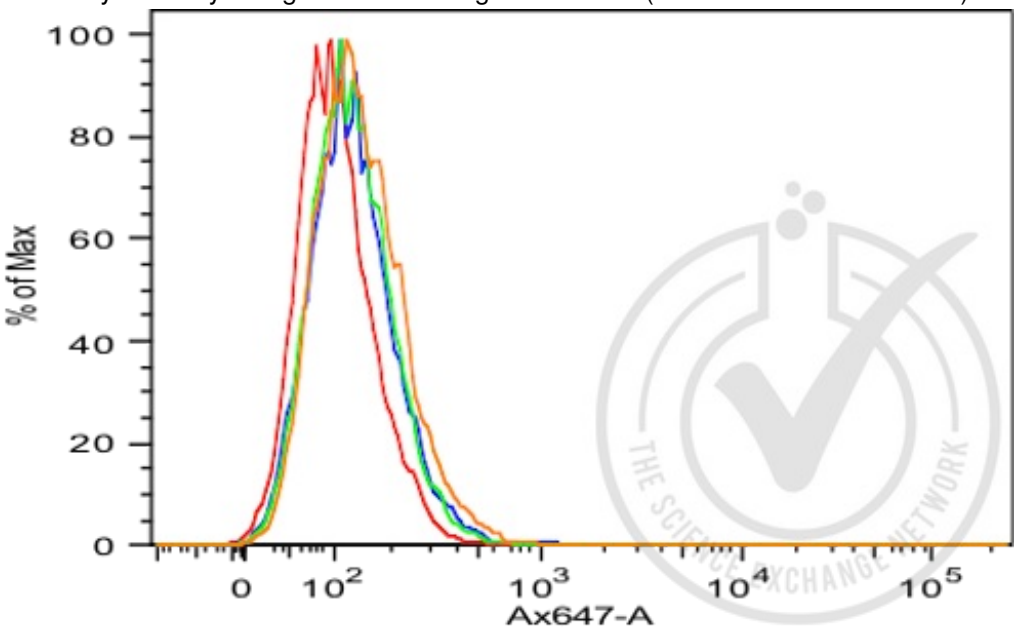

Figure 2: Negative control SH-SY5Y cells. The red histogram is unstained cells, the blue histogram is cells stained with secondary antibody alone, the green histogram is cells stained with rabbit IgG isotype control antibody plus secondary antibody and the orange histogram is cells stained with anti-E-Cadherin plus secondary antibody. The secondary antibody is a goat anti-rabbit IgG-Alexa 647 (Jackson Immunoresearch).