Validation Report #029743

Validation Date: 07/01/14

Summary

Antigen	K-Cadherin (CDH6)
Catalog number	ABIN715286
Supplier	Bioss
Supplier catalog number	bs-5823R
Lot number	130823
Method validated	Flow Cytometry
Laboratory	Flow Cytometry & Cell Separation Facility, Purdue University
Validation number	29743
Positive Control	786-O cells and MCF-7 cells
Notes	As expected, strong signal was observed in the cells stained with anti-K-Cadherin (CDH6) antibody. A small amount of non-specific staining was observed from the isotype and secondary antibody only negative controls compared with unstained cells.

Full Methods

Primary Antibody
- Antigen: K-Cadherin (CDH6) antibody
- Catalog number: ABIN715286
- Supplier: Bioss
- Supplier catalog number: bs-5823R
- Lot number: 130823

Isotype Control Antibody
- Antibody: Rabbit IgG
- Catalog number: 3900S
- Supplier: Cell Signaling Technology

Secondary Antibody
- Antibody: Goat anti-rabbit IgG-Alexa 647
- Supplier: Jackson Immunoresearch
- Catalog number: 712-606-150

Controls
- Positive controls: 786-O cells (human renal cell carcinoma) and MCF-7 cells (human breast cancer)
- 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 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
  - 1 µg primary antibody
  - 1 µg isotype control 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

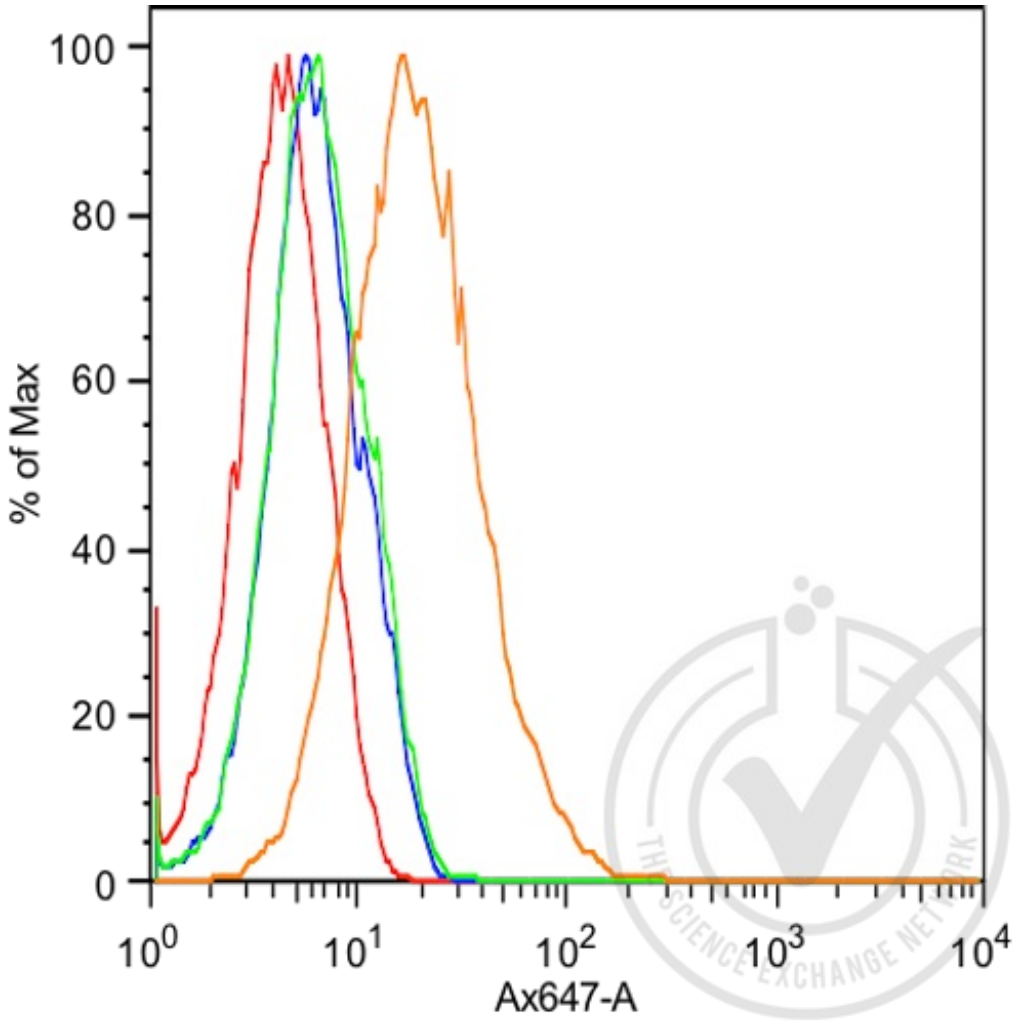

Figure 1. Histogram of 786-O cells stained with anti-K-cadherin (orange), isotype control antibody (green), secondary antibody only (blue) and unstained (red).

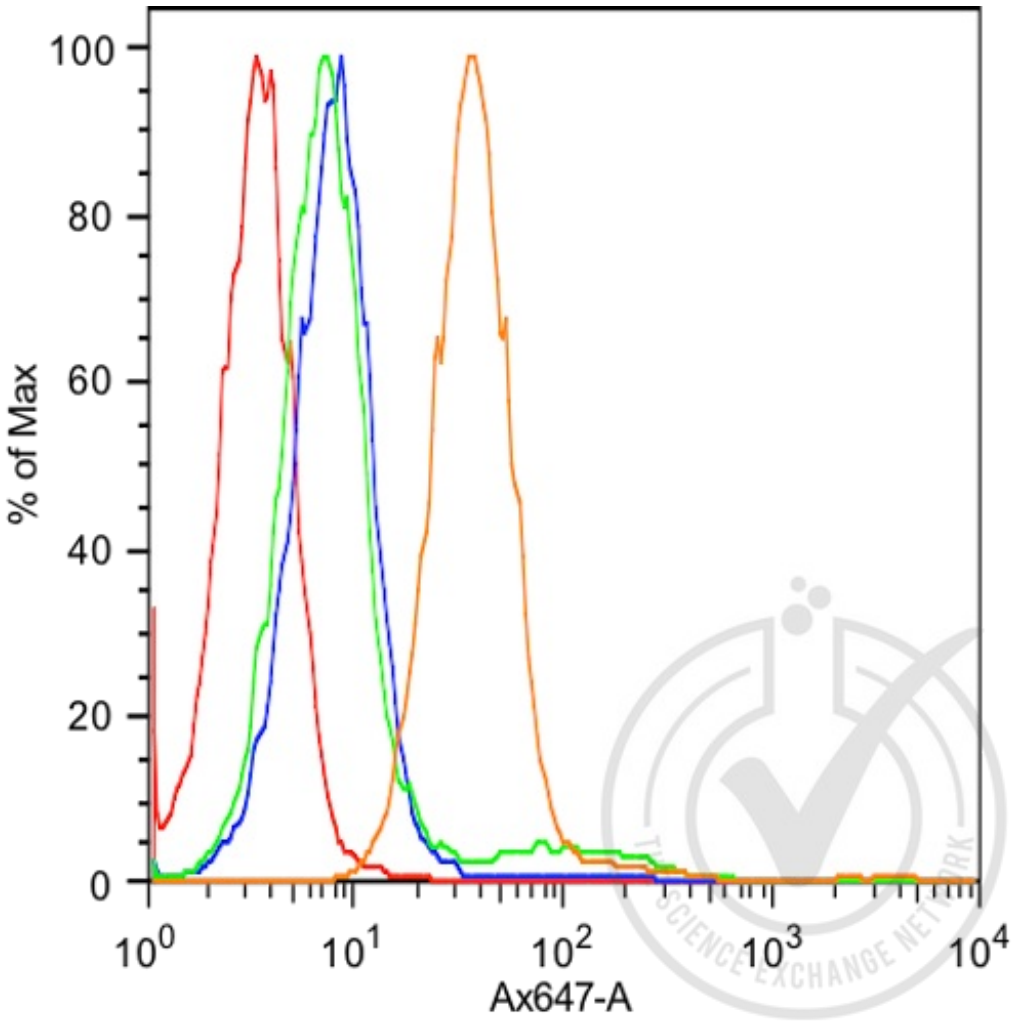

Figure 2. Histogram of MCF-7 cells stained with anti-K-cadherin (orange), isotype control antibody (green), secondary antibody only (blue) and unstained (red).