Validation Report #029824

Validation Date: 11/05/14

Summary

Antigen	Nestin (NES)
Catalog number	ABIN1774766
Supplier	Immuquest
Supplier catalog number	IQ300FITC
Lot number	I030314
Method validated	Immunofluorescence
Laboratory	Ensigna Biosystems
Validation number	029824
Positive Control	PC-3 cells
Negative Control	MCF-7 cells
Notes	Faint fluorescent signal was detected in the positive control cells. No signal was detected in the negative control cells or isotype negative control.

Full Methods

Primary Antibody

- Antigen: Nestin (NES) (FITC)
- Catalog number: ABIN1774766
- Supplier: Immuquest
- Supplier catalog number: IQ300FITC
- Lot number: I030314
- Dilution: 1:75, 1:100

Additional Information

Controls

- Positive control: PC3 Nestin positive prostate adenocarcinoma cells (specimen known to contain high levels the target protein – 100% positive) from ATCC.
- Negative Control: MCF7 Nestin negative breast adenocarcinoma cells (specimen known to contain very low levels of the target protein - <13% positive) from ATCC.

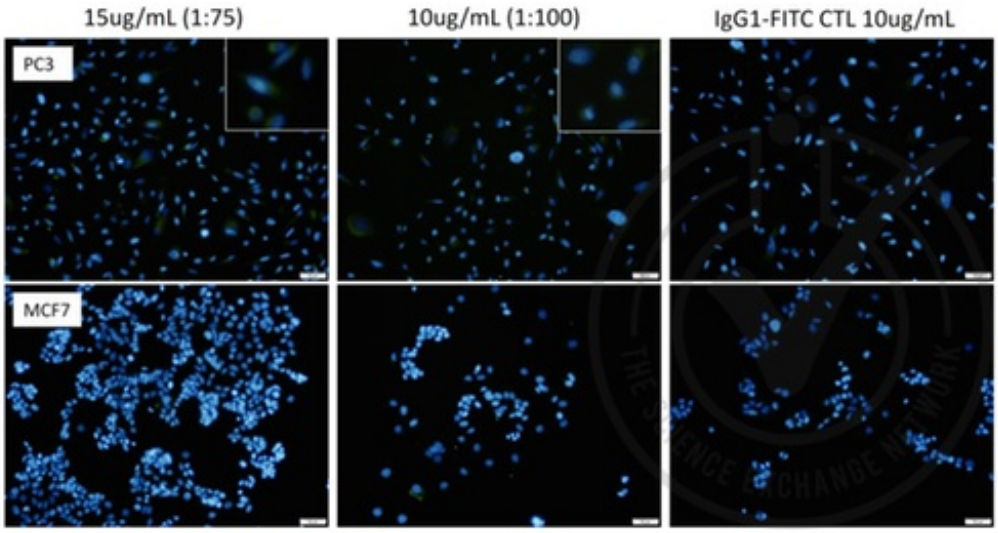
Protocol

- Prostate and breast adenocarcinoma cell lines were grown directly on chamber slides, washed with 1X PBS, and fixed with 4% paraformaldehyde in 1X PBS for 15 min at room temperature (RT).
- Fixed cells were rinsed three times in PBS for 5 min each at RT.
- Cells were blocked in 1X PBS / 5% normal goat serum / 0.3% Triton X-100 for 60 min at RT.
- Cells were rinsed two times in 1X PBS.
- Cells were incubated with primary antibody diluted 1:75 and 1:100 in 1X PBS / 1% BSA / 0.3% Triton X-100 overnight at 4°C in dark conditions.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Coverslips were mounted on slides with DAPI-Fluoromount-G.
- Stained cells were imaged with a Nikon Eclipse E600 microscope and Olympus DP70 camera.

Experimental Notes

The anti-Nestin antibody showed positive staining of weak intensity localized appropriately to the cell cytoplasm in a pattern consistent with intermediate filaments in positive control PC3 cells. No staining was seen in the negative control MCF7 cells or with the isotype control antibody. Although specificity of the antibody was acceptable, its sensitivity with the current protocol was questionable. It is, however, entirely possible that if this antibody is used with an appropriate amplification protocol the sensitivity would be increased.

Figures



Legend: Immunofluorescence. Nestin (green fluorescence) is present in positive control cells (PC3) and absent in negative control cells(MCF7). Scale bar = 50 µm, Magnification = 20X, FITC Exposure = 1.5s, Insets at 40X magnification. Details: Left panel: Micrograph image of PC3 Nestin-positive prostate adenocarcinoma cells (top) and MCF7 Nestin-negative breast adenocarcinoma cells (bottom) with 15 µg/mL of anti-Nestin-FITC Ab. Middle panel: Micrograph image of PC3 Nestin-positive prostate adenocarcinoma cells (top) and MCF7 Nestin-negative breast adenocarcinoma cells (bottom) with 10 µg/mL of anti-Nestin-FITC Ab. Right panel: Micrograph image of reagent isotype control FITC-conjugated mouse IgG1 antibody on PC3 Nestin-positive prostate adenocarcinoma cells (top) and MCF7 breast adenocarcinoma cells (bottom).