

Datasheet for ABIN1774766
anti-Nestin antibody (FITC)



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1 Validation

Overview

Quantity:	50 µL
Target:	Nestin (NES)
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Nestin antibody is conjugated to FITC
Application:	Immunofluorescence (IF), Immunocytochemistry (ICC)

Product Details

Immunogen:	A 150-amino-acid fragment from the cloned human nestin conjugated to GST
Clone:	10C2
Isotype:	IgG1
Specificity:	This antibody recognises nestin, a member of the intermediate filament family required for brain and eye development.
Purification:	Protein G purified

Target Details

Target:	Nestin (NES)
Alternative Name:	Nestin (NES Products)
Background:	Neural Stem Cell Marker

Target Details

Gene ID: 407242

OMIM: 600915

UniProt: [P18175](#)

Application Details

Application Notes: Optimal antibody dilution should be determined by titration.

Restrictions: For Research Use only

Handling

Format: Liquid

Concentration: 1 mg/mL

Buffer: Purified IgG conjugated to Fluorescein using Innova Biosciences Lightning-Link®, supplied in Phosphate buffered saline (PBS) containing 0.09 % Sodium azide

Preservative: Sodium azide

Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.



Successfully validated (Immunofluorescence (IF))

by [Ensigna Biosystems](#)

Report Number: 029824

Date: Nov 06 2014

Lot Number: I030314

Method validated: Immunofluorescence (IF)

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.

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

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.

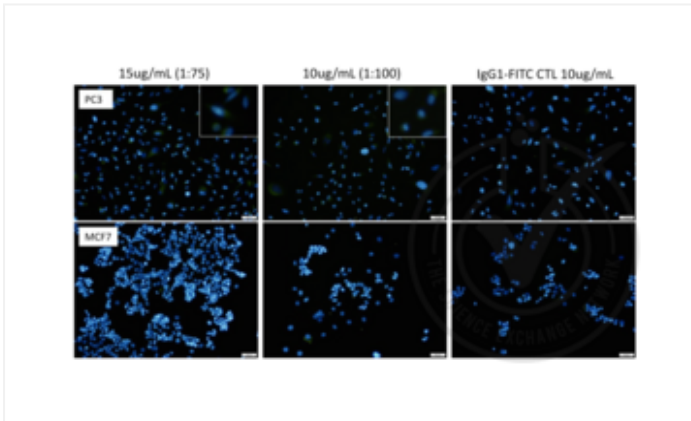
Protocol:

- 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.

Image for Validation report #029824



Validation image no. 1 for anti-Nestin (NES) antibody (FITC) (ABIN1774766)

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).