ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Nestin (Uniprot# P48681)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

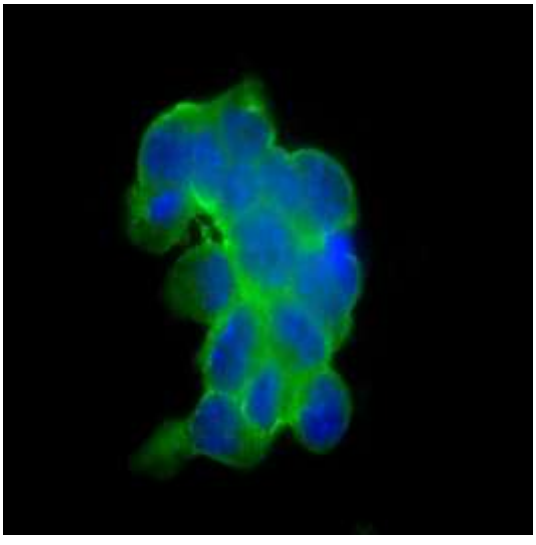
Project ID: AR146

With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

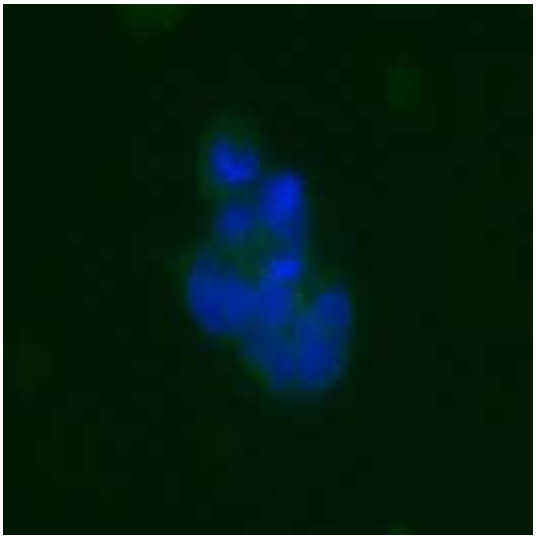
For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

RESULTS

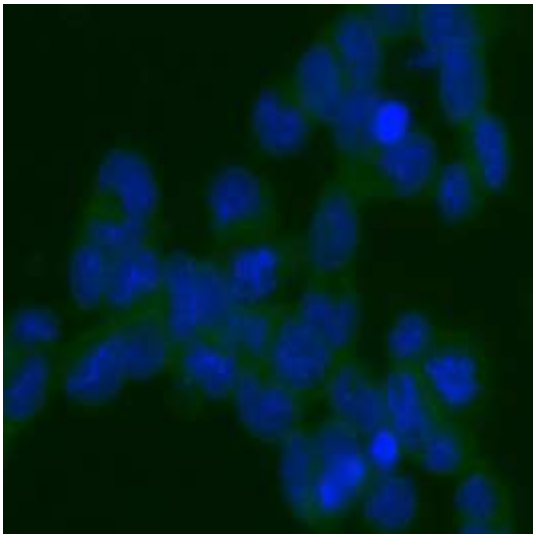
Immunofluoresence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-Nestin antibodies (green) and isotype controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



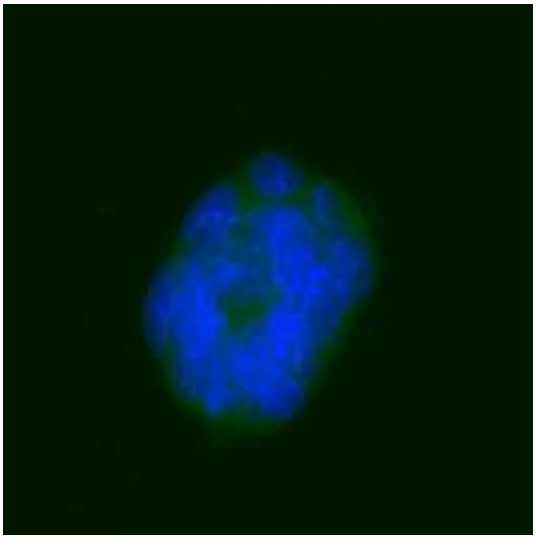
Antibody : Nestin M137 at 1/50
Cell : F9



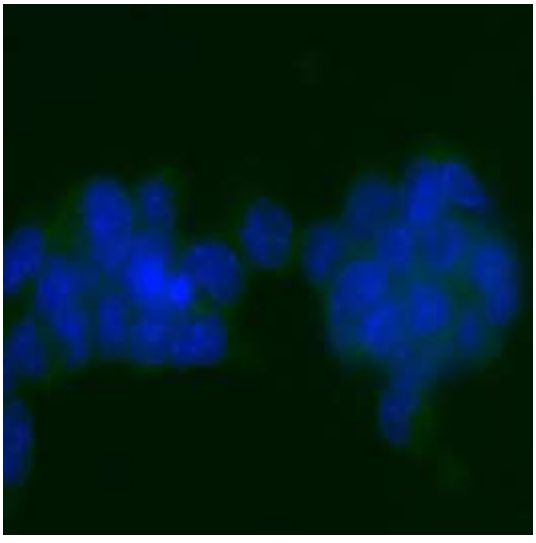
Antibody : Isotype control
Cell : F9



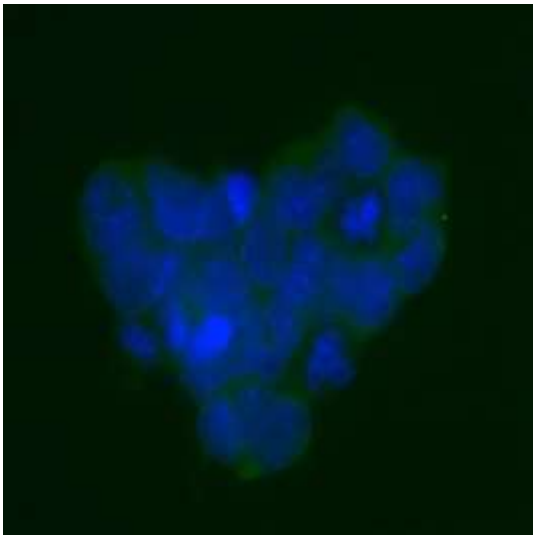
Antibody : Nestin P134 at 1/10000
Cell : F9



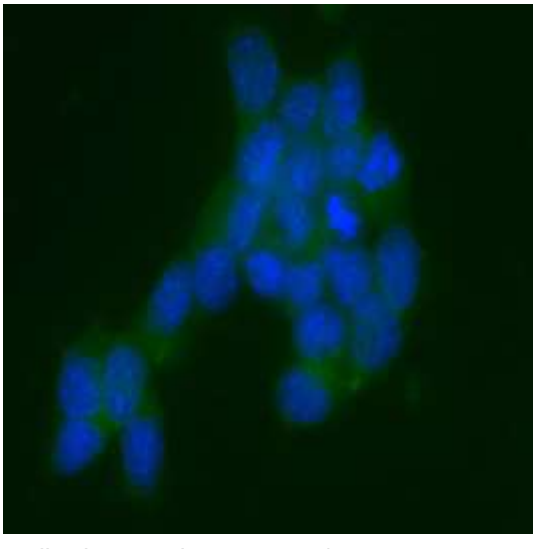
Antibody : Isotype control
Cell : F9



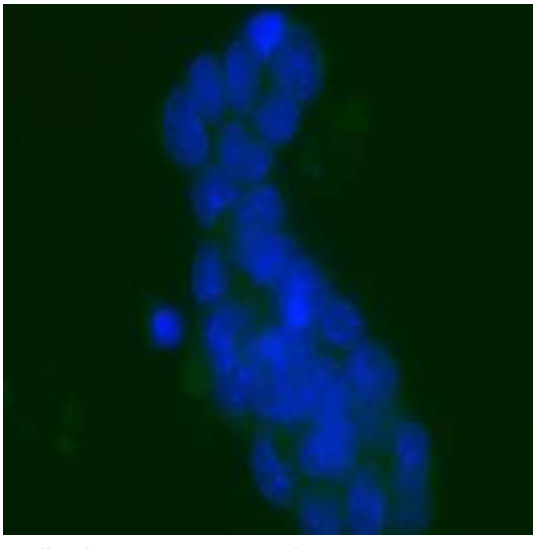
Antibody : Nestin P140 at 1/2000
Cell : F9



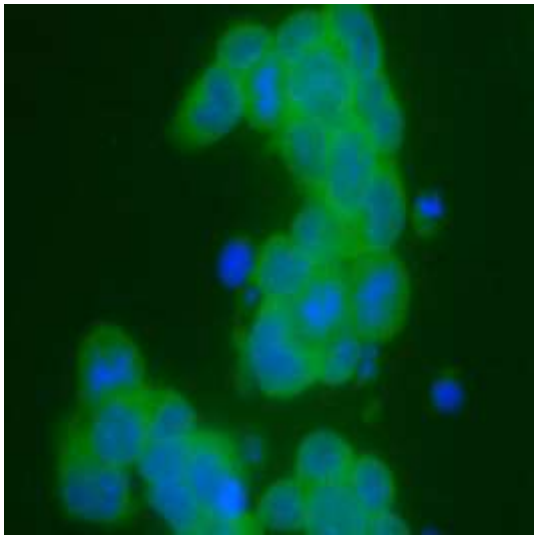
Antibody : Isotype control
Cell : F9



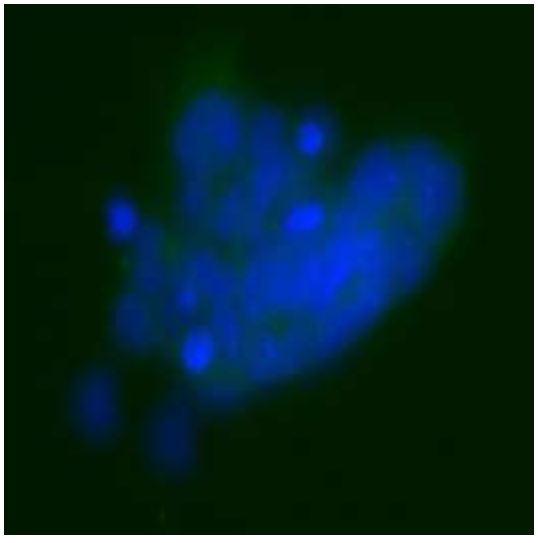
Antibody : Nestin P149 at 1/20
Cell : F9



Antibody : Isotype control
Cell : F9



Antibody : Nestin M175 at 1/100
Cell : F9



Antibody : Isotype control
Cell : F9

METHOD

Antibodies

	Primary antibody	Secondary antibody	Isotype Control
	**Nestin M137 at 1/50 (Novus)**	Donkey anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen, 111357) at 1/200	Mouse IgG1 Isotype Control (ThermoFisher Scientific, MA5-14453) at 1/50
	Nestin P134 at 1/10000 (Supplier 22)	Goat anti-Chicken IgG (IgY)-heavy and light chain cross-adsorbed Antibody DyLight® 488 conjugate (Bethyl, A30-206D2) at 1/200	ChromPure Chicken IgY (IgG), whole molecule (Jackson ImmunoResearch, 003-000-003) at 1/10000
	Nestin P140 at 1/2000 (Supplier 08)	Goat anti-Chicken IgG (IgY)-heavy and light chain cross-adsorbed Antibody DyLight® 488 conjugate (Bethyl Laboratories, Inc, A30-206D2) at 1/200	ChromPure Chicken IgY (IgG), whole molecule (Jackson ImmunoResearch, 003-000-003) at 1/2000
	Nestin P149 at 1/20 (Supplier 31)	Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (ThermoFisher Scientific, A-11008) at 1/200	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/20
	Nestin M175 at 1/100 (BioLegend)	Goat anti-Rat IgG-heavy and light chain cross-adsorbed Antibody DyLight® 488 conjugate (Bethyl Laboratories, Inc, A110-305D2) at 1/200	ChromPure Rat IgG, whole molecule (Jackson ImmunoResearch, 012-000-003) at 1/100

 = Component of the Nestin Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunofluorescent analysis of F9 cells (Mouse teratocarcinoma) was performed using a LEICA TCS SP2 Confocal Laser Scanning Microscope. Cells were prepared prior to analysis as follows:-

1. Cells, grown in 12 multiwell plates, were washed twice in 0.5 ml of PBS per well at room temperature for 5 minutes per wash.
2. The cells were then fixed in 4% formalin by adding 0.2ml of the formalin solution to each well for 20 minutes at room temperature. After removal of the 4% formalin, the cells were washed twice in PBS as described above.
3. Penetration of the cells was then performed by adding 0.2ml of 0.1% Triton X-100 per well for 10 minutes at room temperature.
4. Following washing with PBS as described above, a blocking step was performed by adding 0.3ml of 0.3% BSA in PBS to each well for 30 minutes at room temperature.
5. After removal of the blocking solution, the cells were incubated with 0.3ml primary antibody or control diluted in 0.3% BSA in PBS (for details see table above) for 60 minutes at 37^{o}C.
6. Following removal of the primary antibody/control solution and three washes for 5 minutes each with PBST, the cells were incubated in 0.3ml per well of secondary antibody diluted in 0.3% BSA PBS (for details see table above) for 50 minutes at 37^{o}C and protected from light.
7. The cells were washed three times with PBST and nuclei staining performed by adding 0.2ml of 10µg/ml DAPI solution to each well for 10 minutes at room temperature. After removal of the DAPI solution, the cells were washed with PBST as described previously.
8. The cells were then transferred to a clean glass slide and fluoromount mounting medium added. A coverslip was carefully placed onto the slide, using absorbant paper to remove excess liquid and avoiding bubble formation.
9. The cells were observed and the results recorded.

EXPERIMENTAL NOTES

Under these experimental conditions, Nestin M137 and Nestin M175 exhibit immunofluorescent staining of Mouse F9 cells consistent with the expected cellular location. Neither Nestin P134, Nestin P140 or Nestin P149 demonstrate staining as compared to isotype controls.

SUPERSTARTER ANTIBODY PANELS

A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only $363, €350, £259.

The TNF-α Superstarter Antibody Panel consists of:

1 x	AF2736	(R&D Systems)
1 x	MAB353	(Millipore)
1 x	NB300-266	(Novus Biologicals)

http://www.antibodyresource.com/superstars

Images of Superstar Nestin antibodies: