

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Vimentin (Uniprot# P08670)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

Project ID: AR128

BACKGROUND

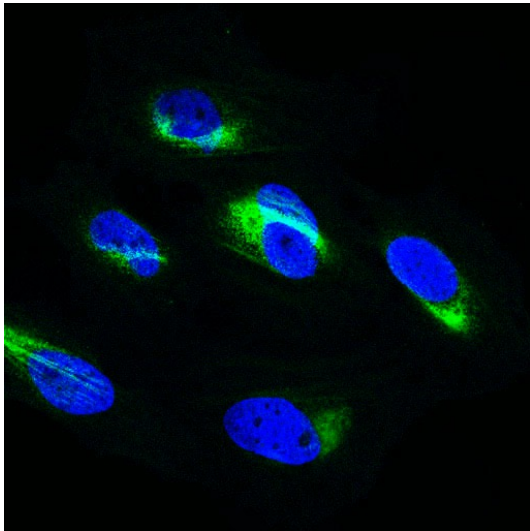
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical 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.

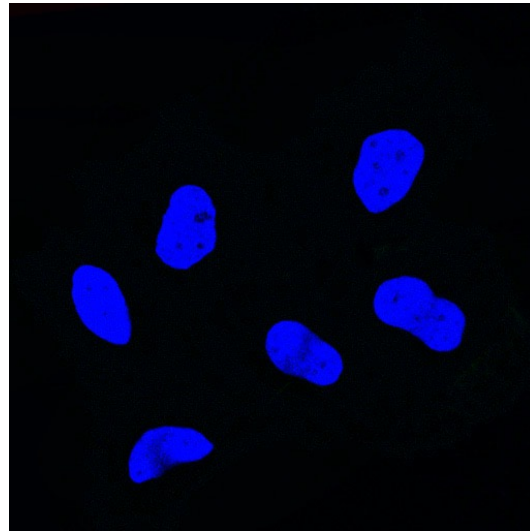
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

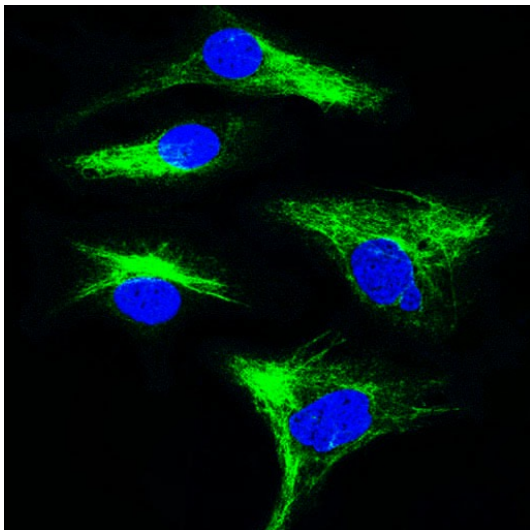
Immunofluorescence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-Vimentin antibodies (green) and controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



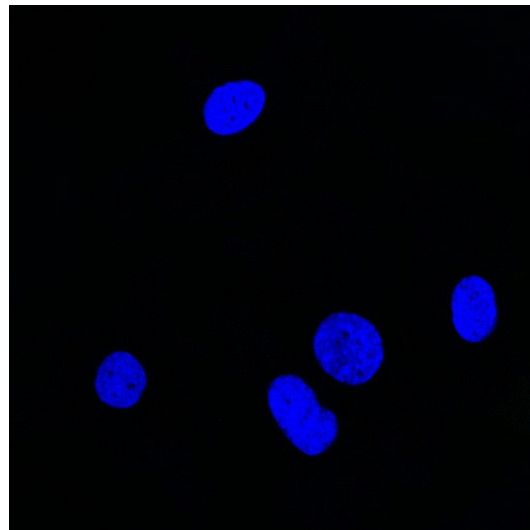
Antibody : [VIM M22 at 1/100 \(Progen\)](#)
Cell : HeLa



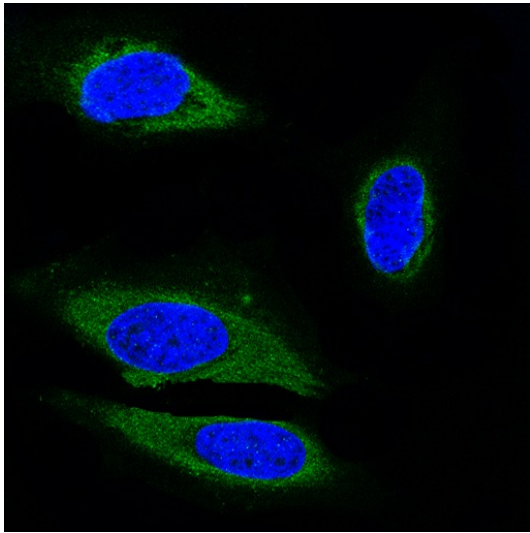
Antibody : Isotype control
Cell : HeLa



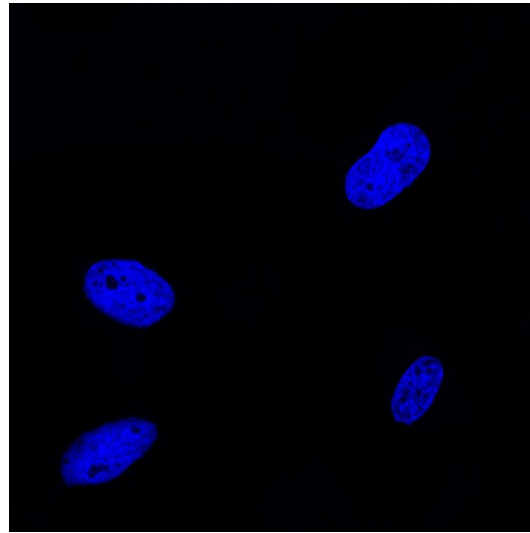
Antibody : [VIM M22 at 1/100 \(Progen\)](#)
Cell : SKOV-3



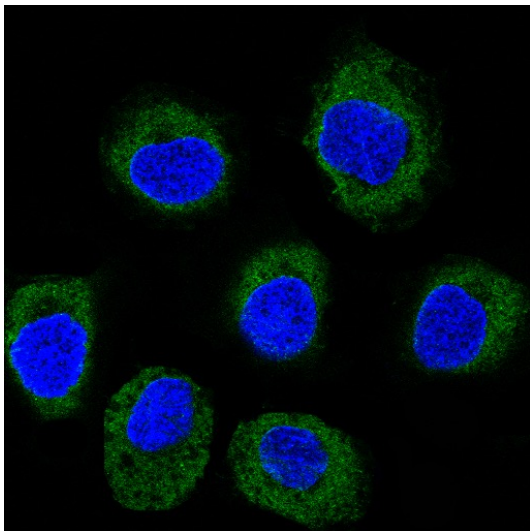
Antibody : Isotype control
Cell : SKOV-3



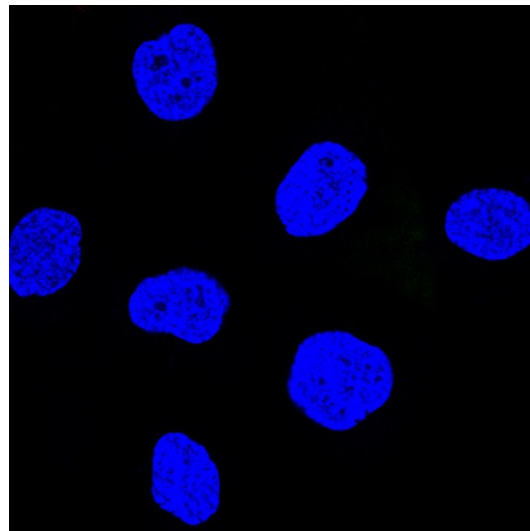
Antibody : VIM P21 at 1/1000 (Supplier 08)
Cell : HeLa



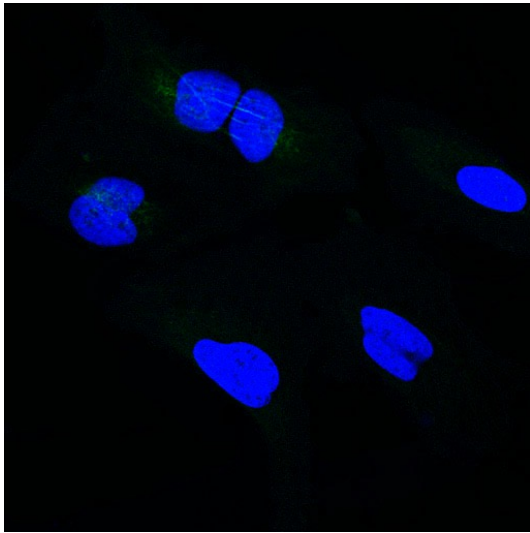
Antibody : Negative control
Cell : HeLa



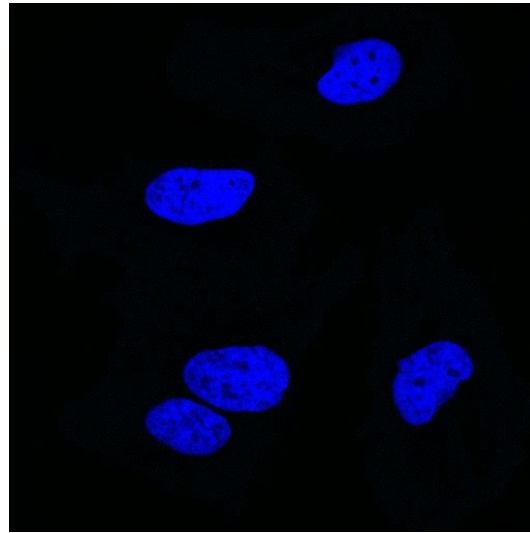
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Cell : SKOV-3



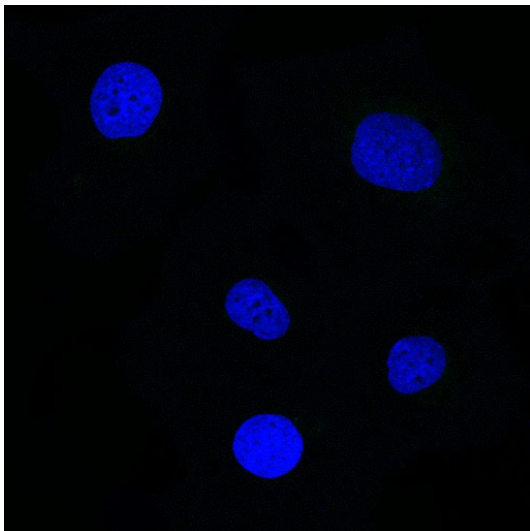
Antibody : Negative control
Cell : SKOV-3



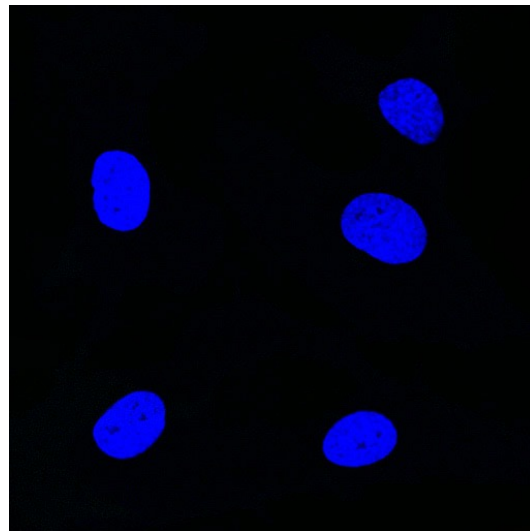
Antibody : VIM M23 at 1/100 (Supplier 15)
Cell : HeLa



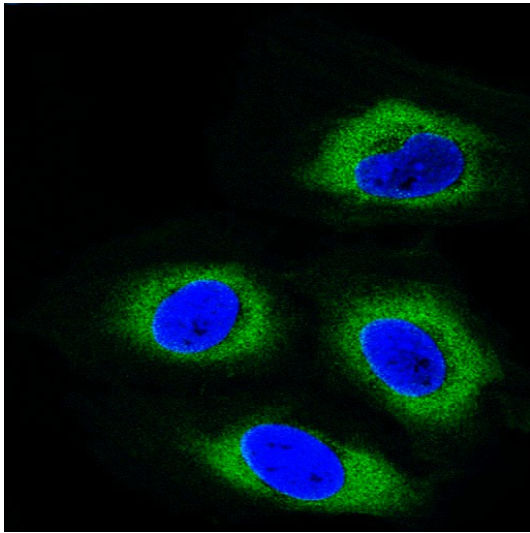
Antibody : Isotype control
Cell : HeLa



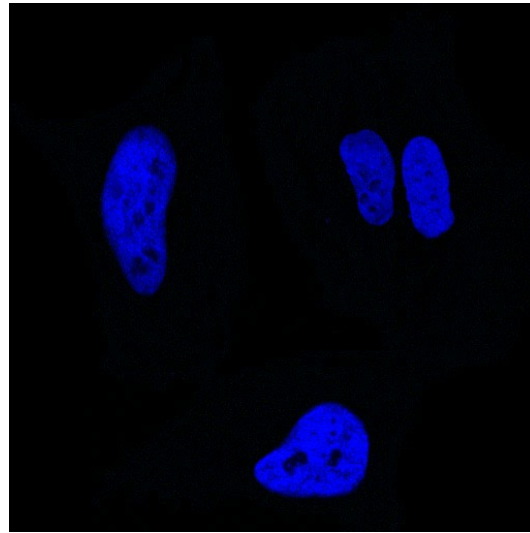
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Cell : SKOV-3



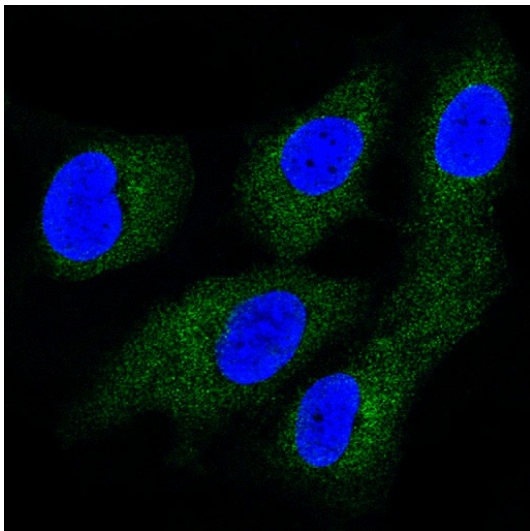
Antibody : Isotype control
Cell : SKOV-3



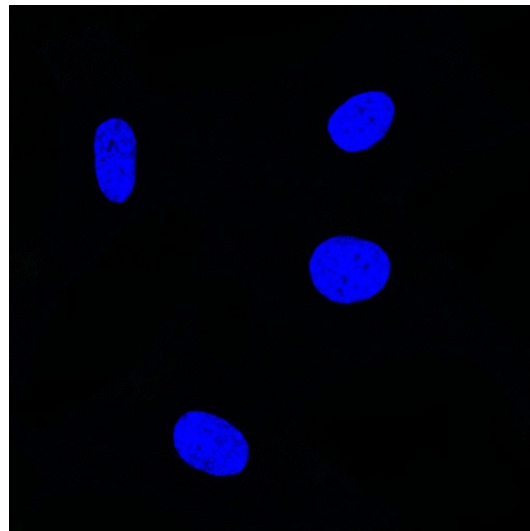
Antibody : VIM P22 at 1/100 (Supplier 29)
Cell : HeLa



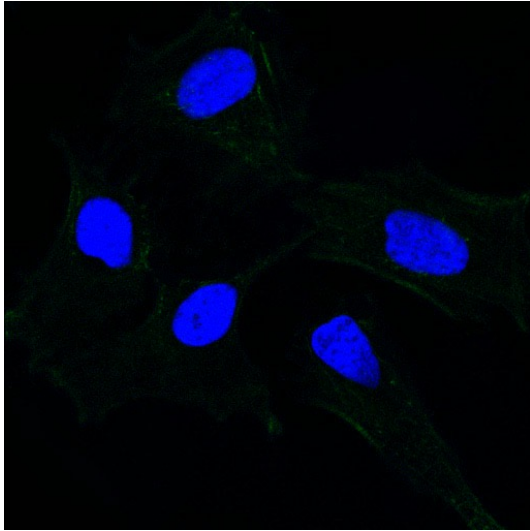
Antibody : Isotype control
Cell : HeLa



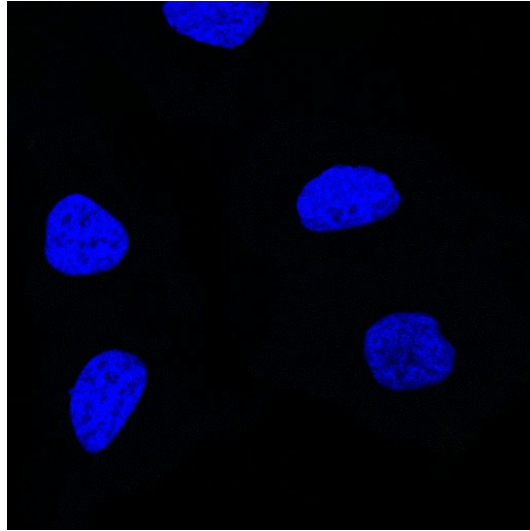
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Cell : SKOV-3



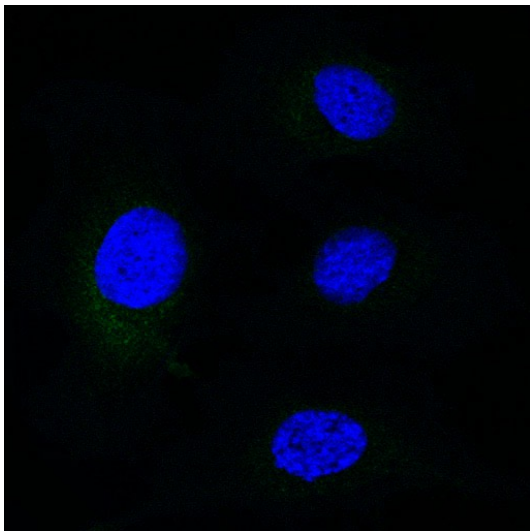
Antibody : Isotype control
Cell : SKOV-3



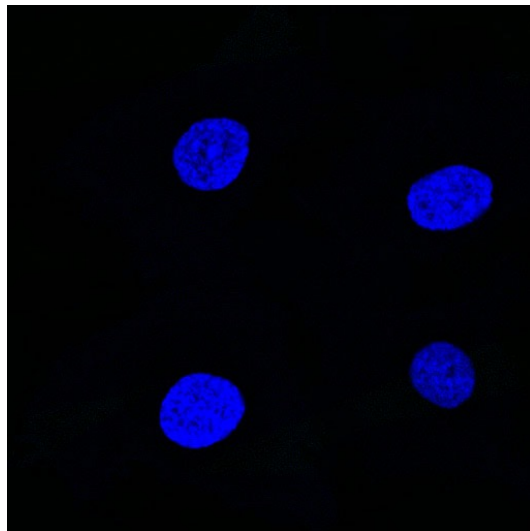
Antibody : VIM M24 at 1/100 (Supplier 07)
Cell : HeLa



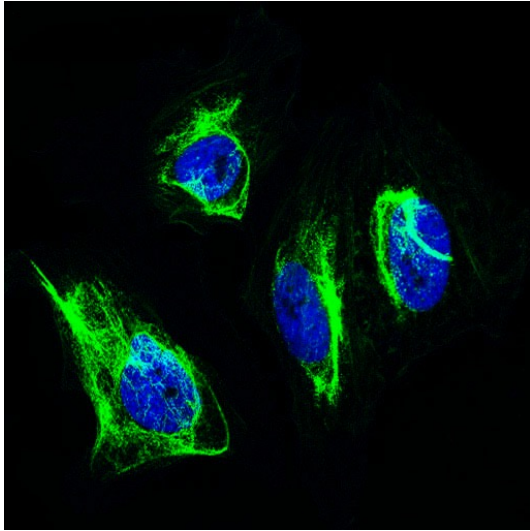
Antibody : Isotype control
Cell : HeLa



Antibody : VIM M24 at 1/100 (Supplier 07)
Cell : SKOV-3

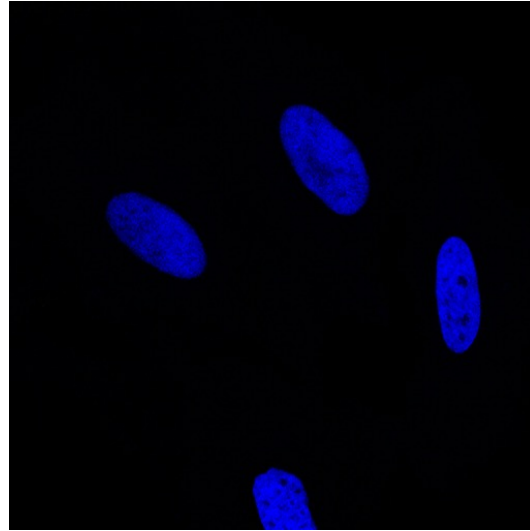


Antibody : Isotype control
Cell : SKOV-3



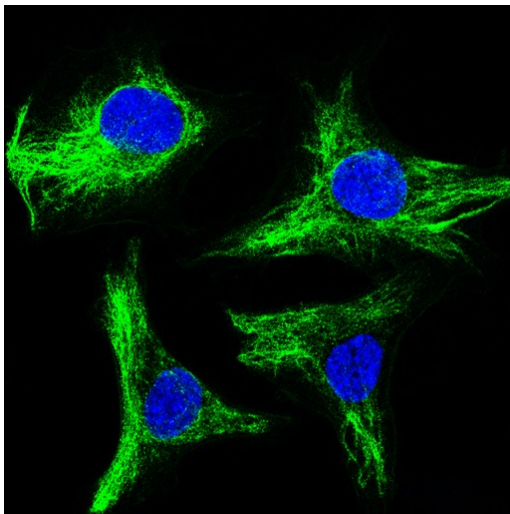
Antibody : [VIM P23 at 1/100 \(Atlas\)](#)

Cell : HeLa



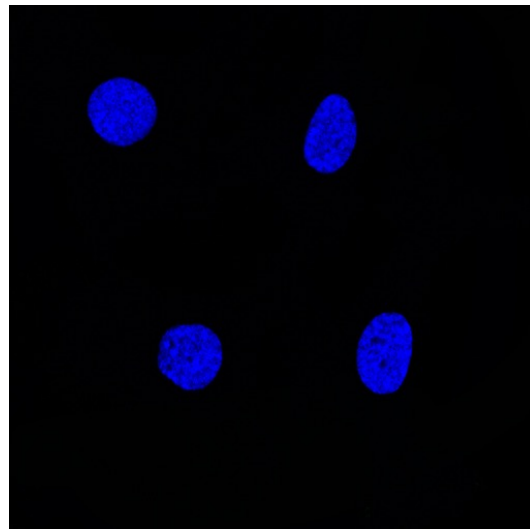
Antibody : Isotype control

Cell : HeLa



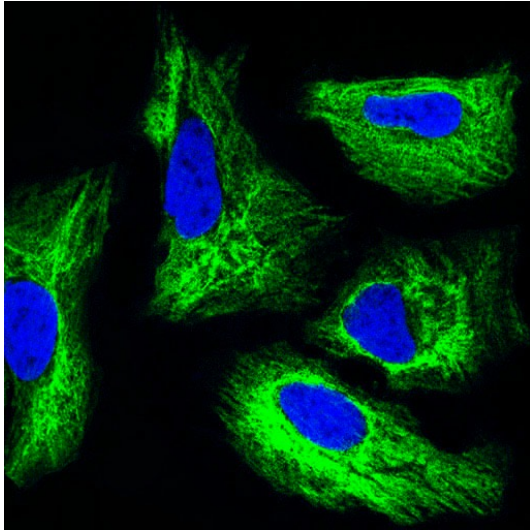
Antibody : [VIM P23 at 1/100 \(Atlas\)](#)

Cell : SKOV-3

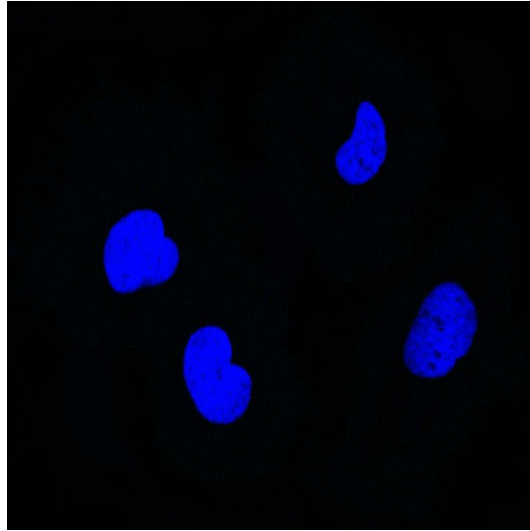


Antibody : Isotype control

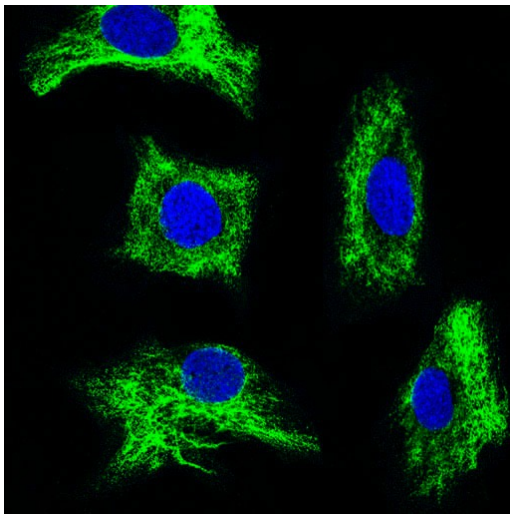
Cell : SKOV-3



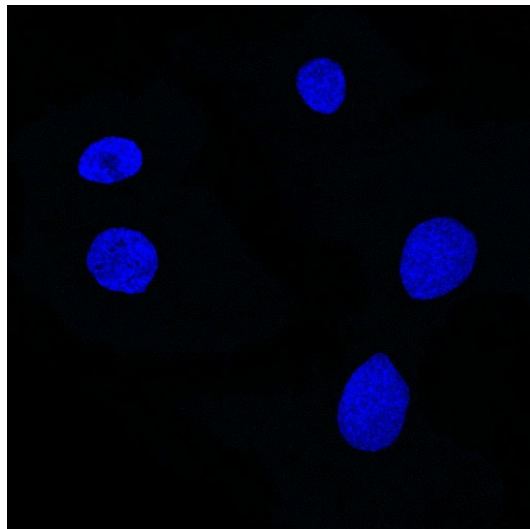
Antibody : [VIM M25 at 1/100 \(Acris\)](#)
Cell : HeLa



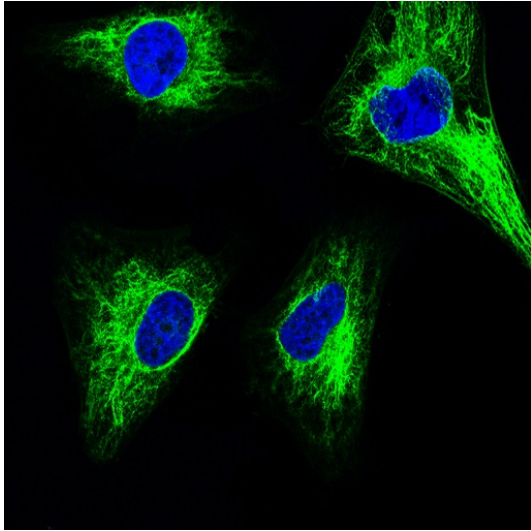
Antibody : Isotype control
Cell : HeLa



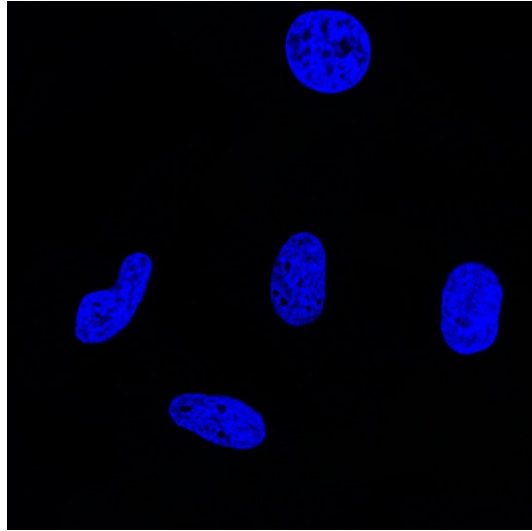
Antibody : [VIM M25 at 1/100 \(Acris\)](#)
Cell : SKOV-3



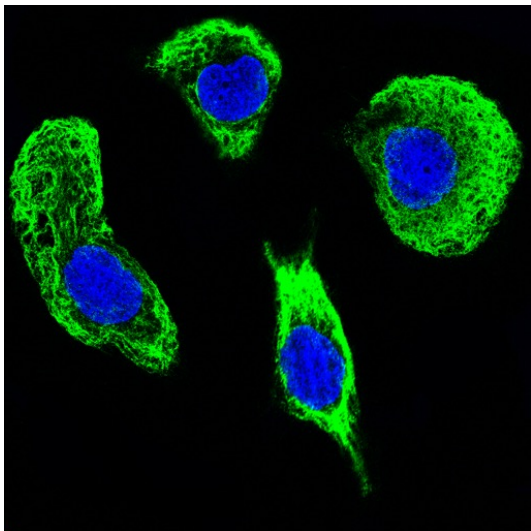
Antibody : Isotype control
Cell : SKOV-3



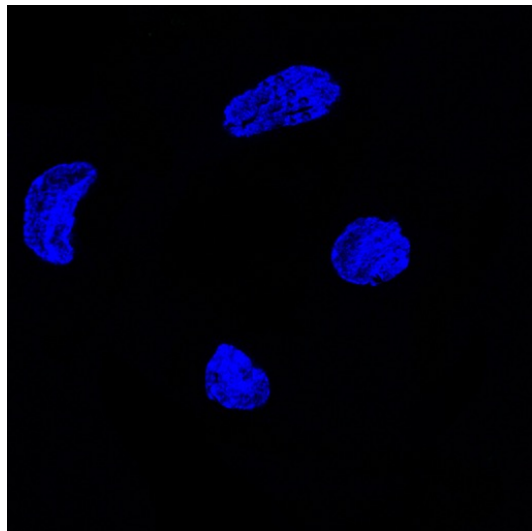
Antibody : [VIM P24 at 1/5000 \(Novus\)](#)
Cell : HeLa



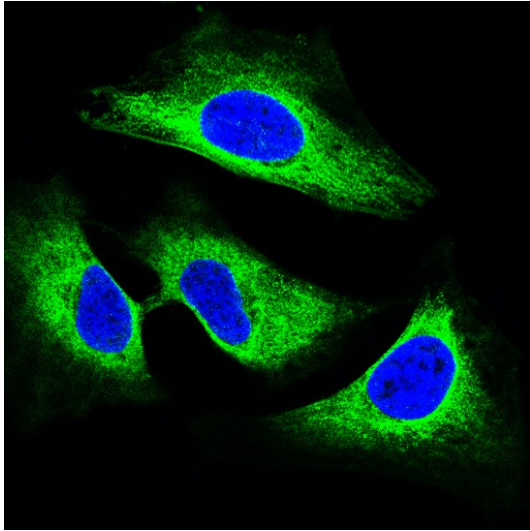
Antibody : Negative control
Cell : HeLa



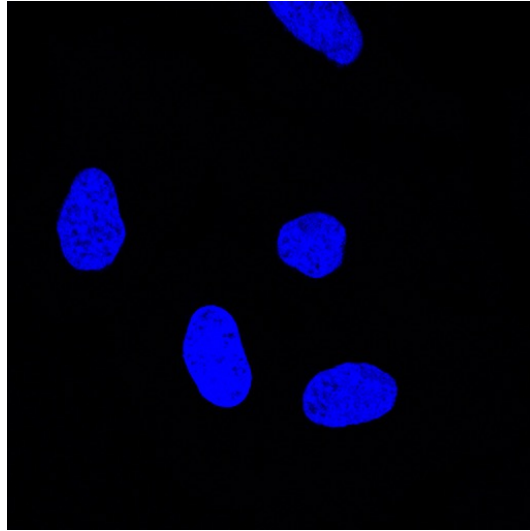
Antibody : [VIM P24 at 1/500 \(Novus\)](#)
Cell : SKOV-3



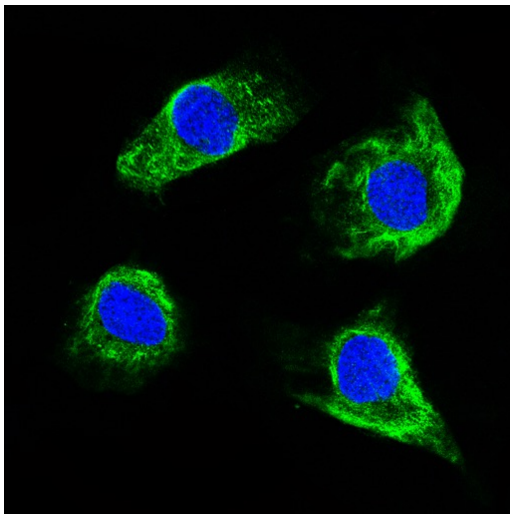
Antibody : Negative control
Cell : SKOV-3



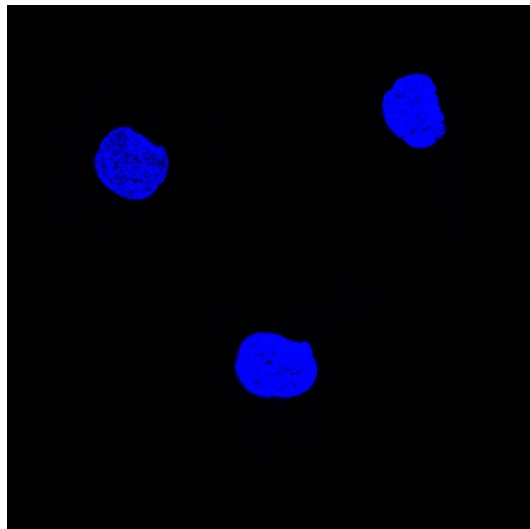
Antibody : VIM M31 at 1/100 (Supplier 16)
Cell : HeLa



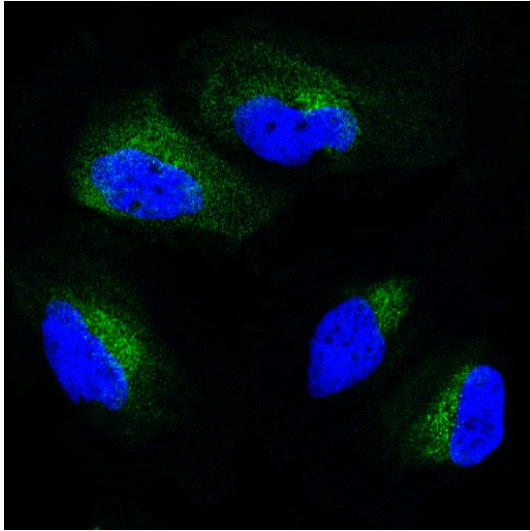
Antibody : Negative control
Cell : HeLa



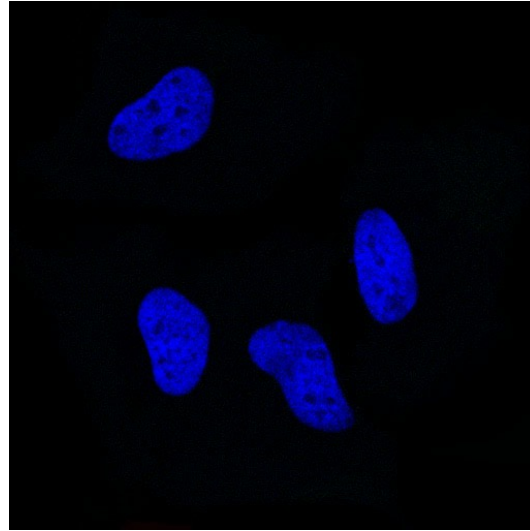
Antibody : VIM M31 at 1/100 (Supplier 16)
Cell : SKOV-3



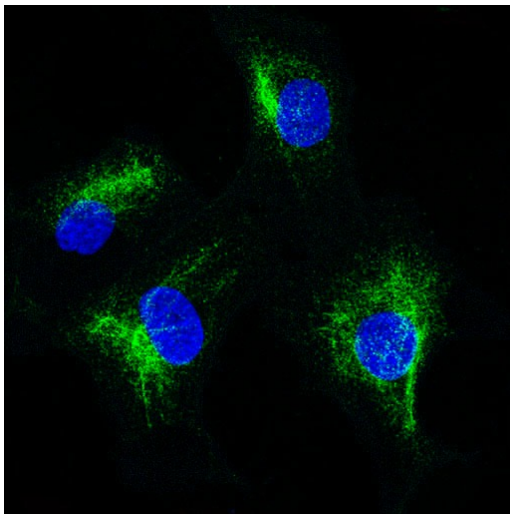
Antibody : Negative control
Cell : SKOV-3



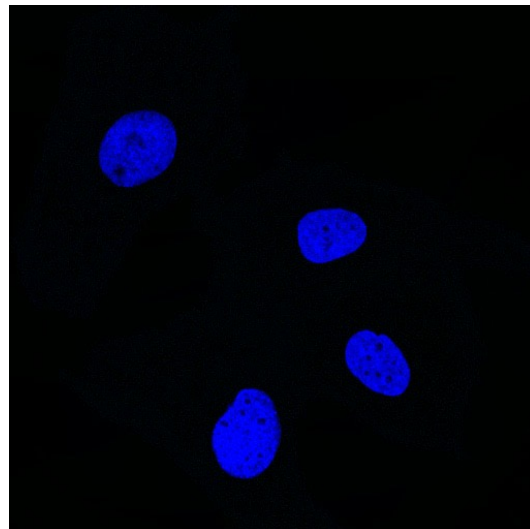
Antibody : [VIM M99 at 1/100 \(Cell Signalling\)](#)
Cell : HeLa



Antibody : Isotype control
Cell : HeLa



Antibody : [VIM M99 at 1/100 \(Cell Signalling\)](#)
Cell : SKOV-3




Antibody : Isotype control
Cell : SKOV-3

METHOD

Antibodies

	Primary antibody	Secondary antibody	Control
	VIM M22 at 1/100 (Progen)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG2a Isotype Control (Thermo Scientific, MA5-14441) at 1/100
	VIM P21 at 1/1000 (Supplier 08)	Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson ImmunoResearch, 703-545-155) at 1/200	PBS
	VIM M23 at 1/100 (Supplier 15)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/100
	VIM P22 at 1/100 (Supplier 29)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35553) at 1/200	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 1/100
	VIM M24 at 1/100 (Supplier 07)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/100
	VIM P23 at 1/100 (Atlas)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35553) at 1/200	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 1/100
	VIM M25 at 1/100 (Acris)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgM Isotype Control (Thermo Scientific, MA1-10438) at 1/100
	VIM P24 at 1/5000 (Novus)	Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson ImmunoResearch, 703-545-155) at 1/200	PBS
	VIM M31 at 1/100 (Supplier 16)	Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L) (Jackson ImmunoResearch, 706-545-148) at 1/200	PBS

	VIM M99 at 1/100 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35553) at 1/200	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 1/100
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 = Component of the Vimentin Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunofluorescent analysis of HeLa cells (Human epithelial cells from cervix adenocarcinoma) and SKOV-3 cells (Human epithelial cells from ovarian carcinoma) 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°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°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.

EXPERIMENTAL NOTES

Vimentin is an intermediate filament protein. Under these experimental conditions, VIM P23, VIM P24 and VIM M25 exhibit staining on both cells types consistent with this cellular location. VIM P21 and VIM P22 show cytoplasm staining of these cells whilst no staining of either cell type is seen using VIM M23 or VIM M24. The immunofluorescent staining using VIM M22 and VIM M99 suggests intermediate filament binding on the SKOV-3 cells but requires confirmation on HeLa cells as the binding could be interpreted as cytoplasm staining.

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 \$240, €240, £178.

The Vimentin Superstarter Antibody Panel consists of:

- 1x Cell Signaling Technology [5471](#) (high reviews)
- 1x Santa Cruz Biotechnology [sc-6260](#) (high reviews)
- 1x Sigma-Aldrich [V6630](#) (star performer)

<http://www.antibodyresource.com/superstars>

Images of Superstar Vimentin antibodies:

