ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: CD31 (Uniprot# P16284)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

Project ID: AR123

BACKGROUND

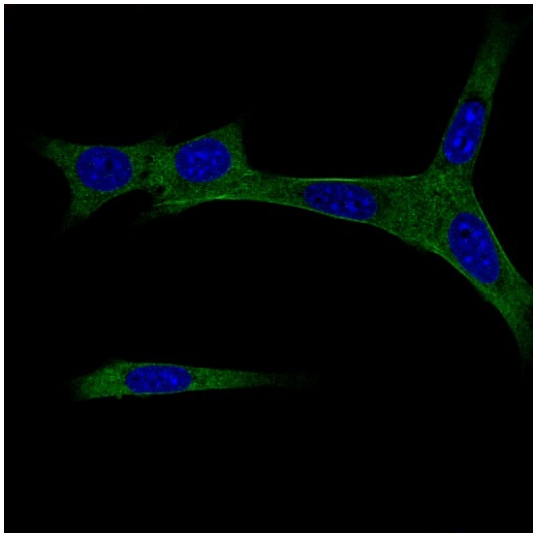
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical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

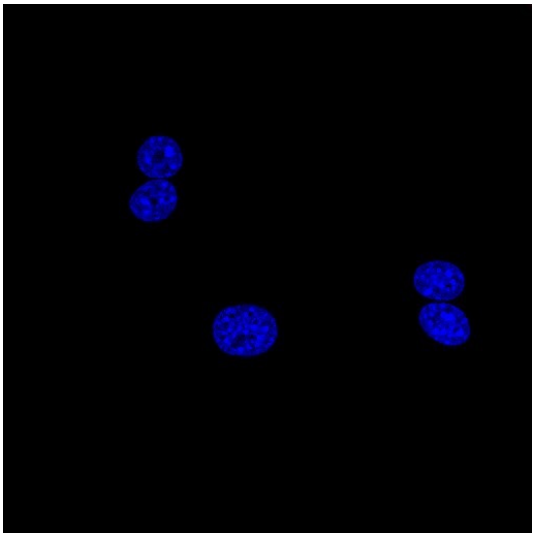
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

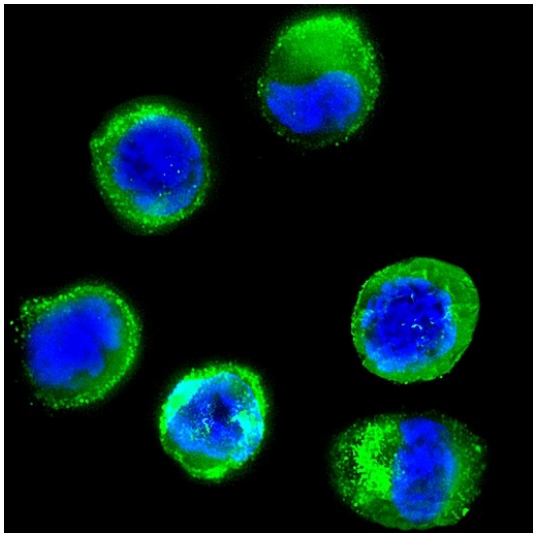
Immunofluoresence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-CD31 antibodies (green) and isotype controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



Antibody : CD31 M31 at 1/50 (Supplier 15)
Cell : NIH/3T3



Antibody : Isotype control
Cell : NIH/3T3



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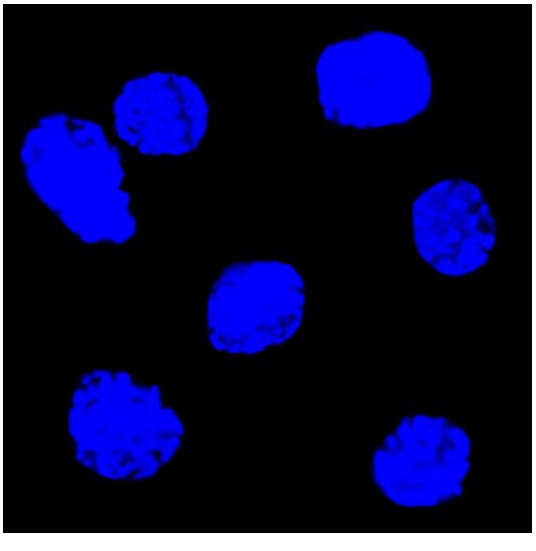
Cell : THP-1



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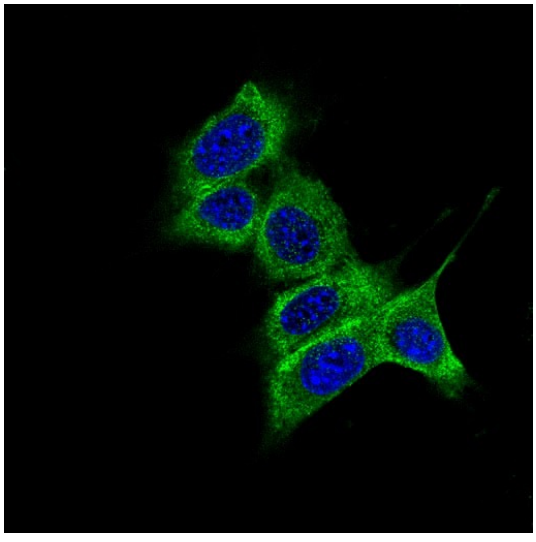

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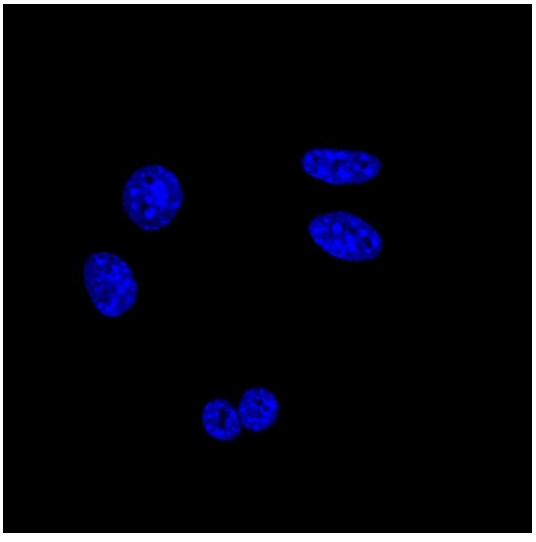

Antibody : Isotype control
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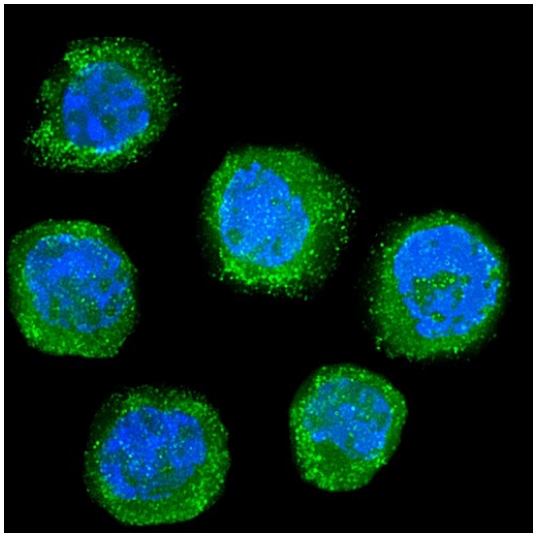
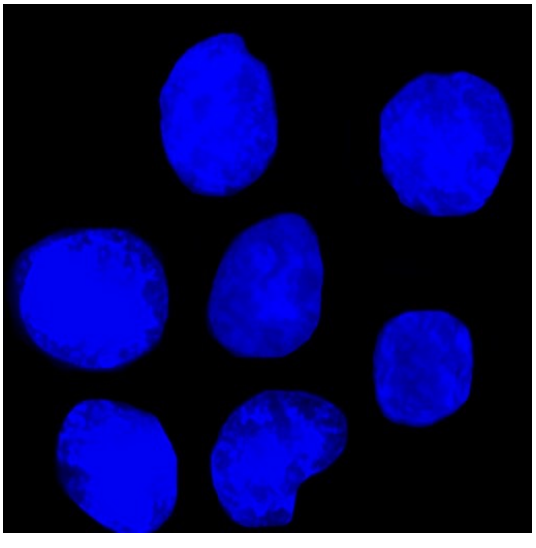


Antibody : CD31 M32 at 1/50 (Supplier 06)
Cell : THP-1



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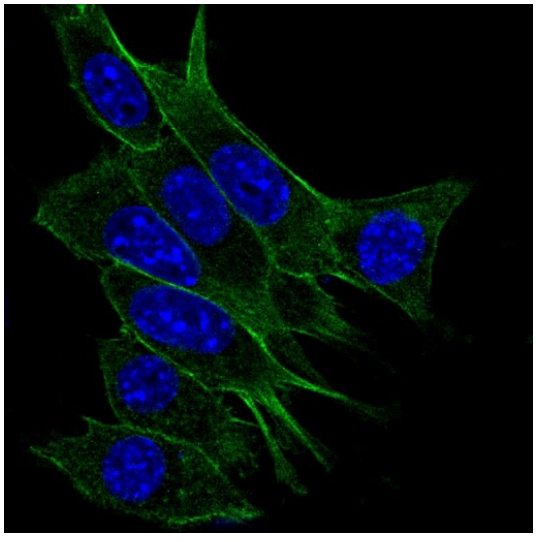
Cell : THP-1



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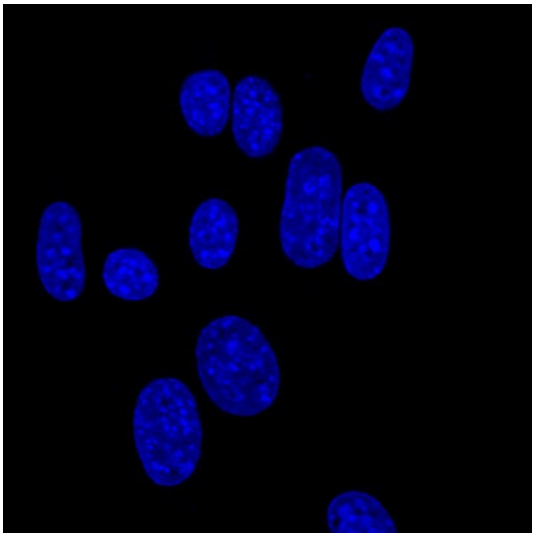
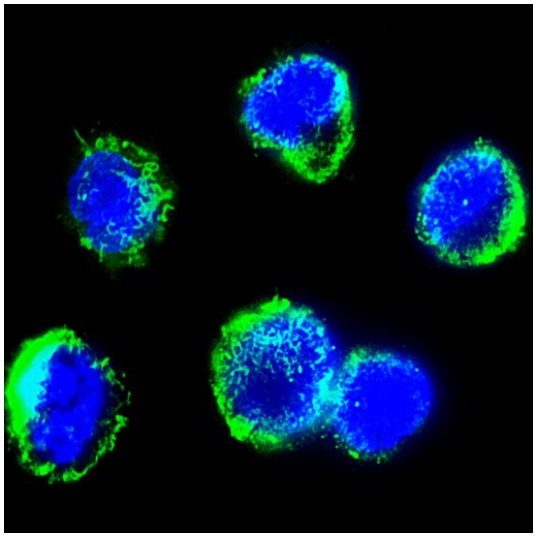
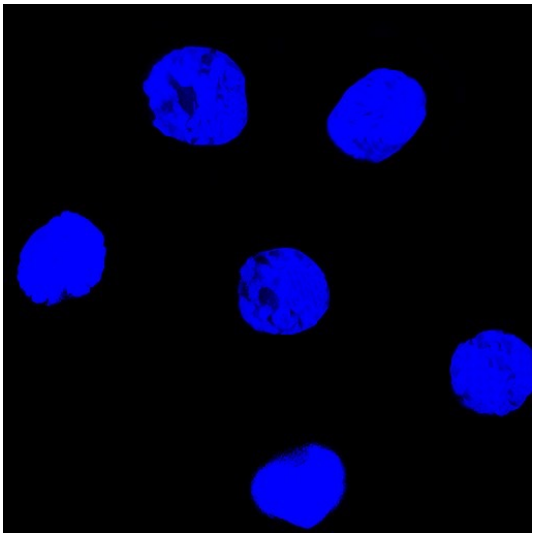


Antibody : Isotype control
Cell : NIH/3T3



Antibody : CD31 M33 at 1/10 (Novus)
Cell : THP-1



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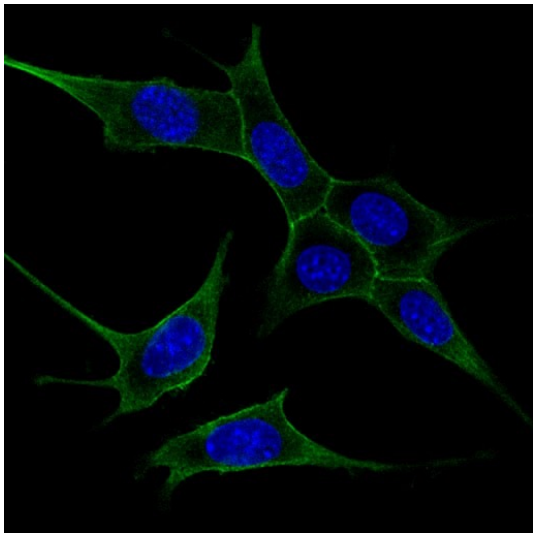
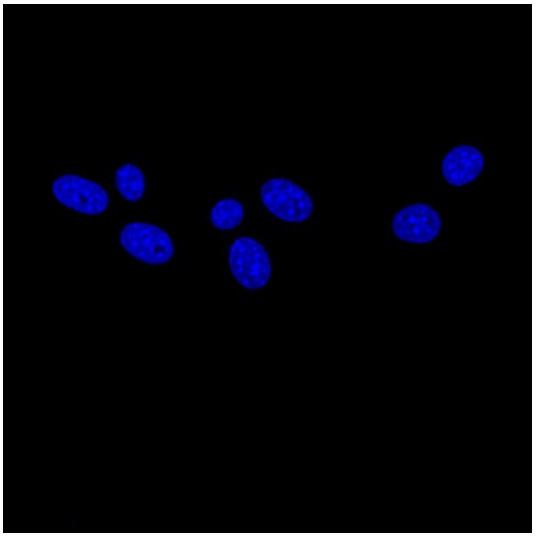
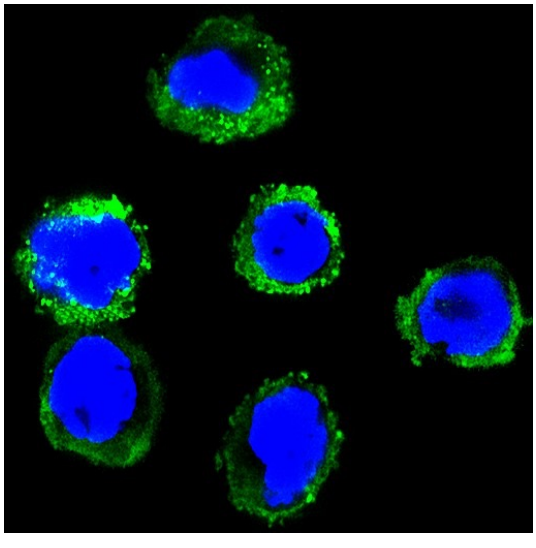
Cell : THP-1



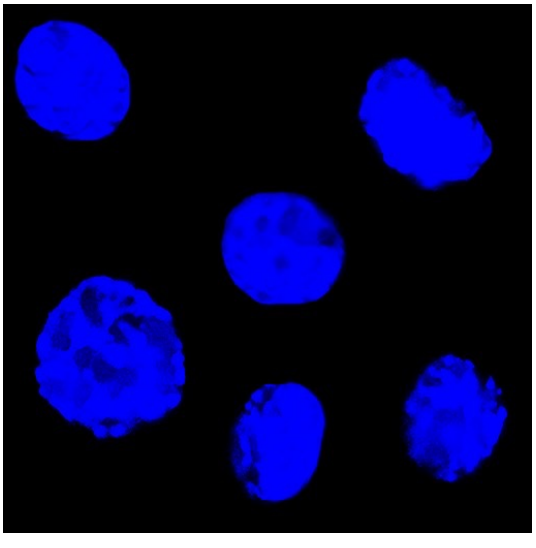
Antibody : CD31 M34 at 1/500 (Fitzgerald)
Cell : NIH/3T3



Antibody : Isotype control
Cell : NIH/3T3



Antibody : CD31 M34 at 1/500 (Fitzgerald)
Cell : THP-1



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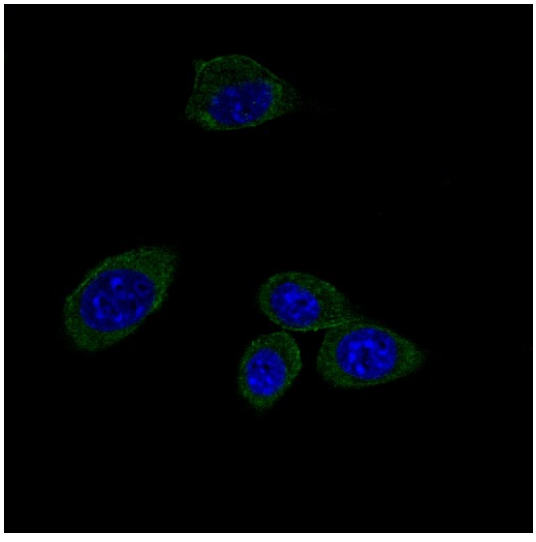
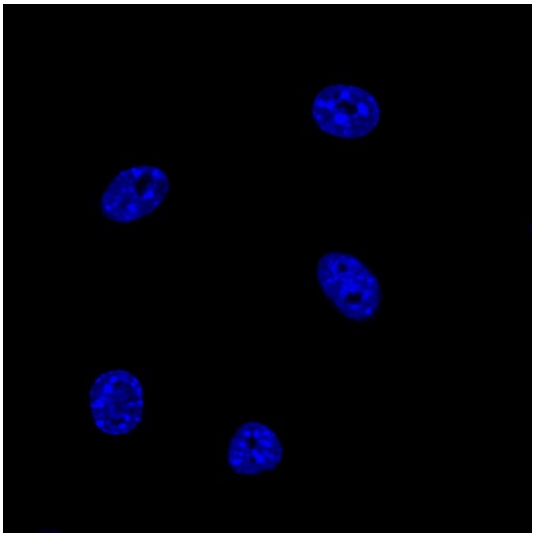
Cell : THP-1



Antibody : CD31 P33 at 1/500 (Supplier 29)
Cell : NIH/3T3



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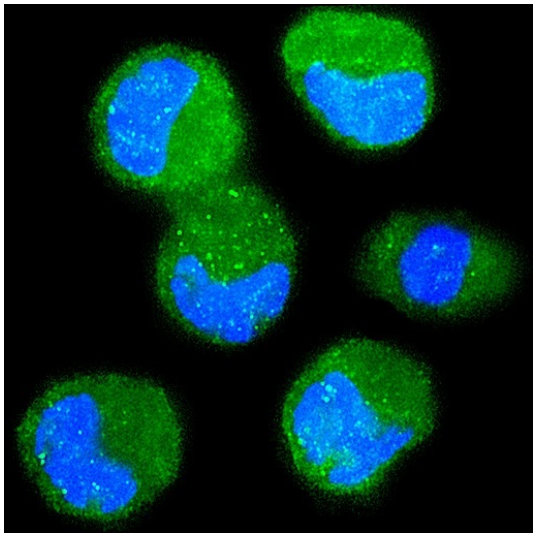

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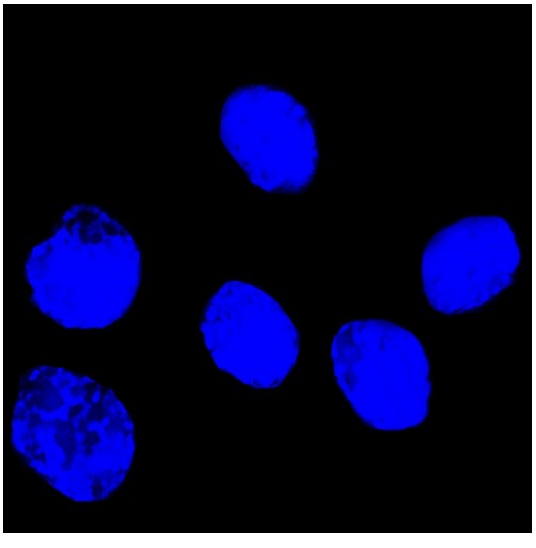

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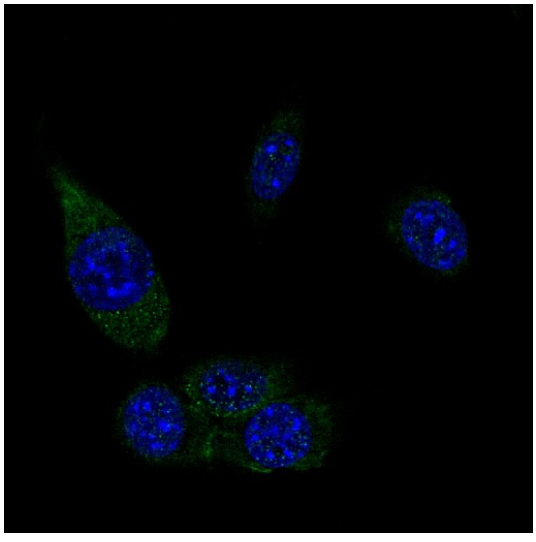
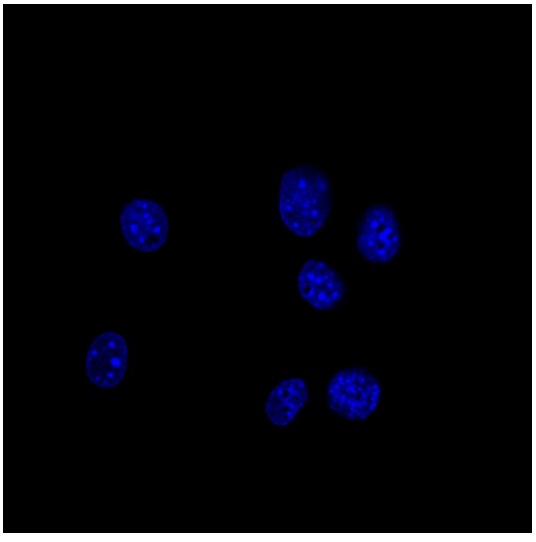


Antibody : CD31 P34 at 1/500 (Supplier 11)
Cell : NIH/3T3



Antibody : Isotype control
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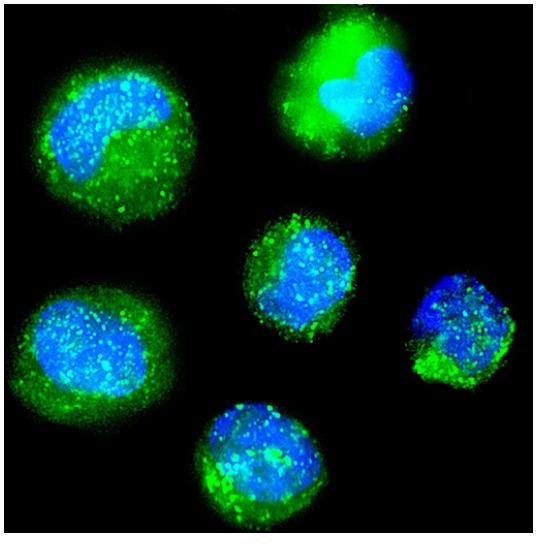

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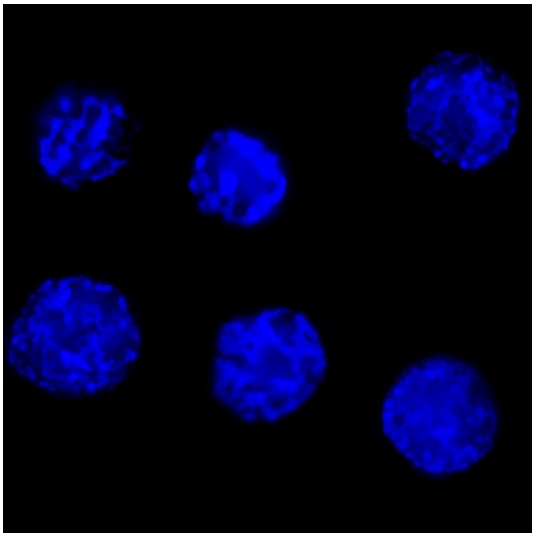

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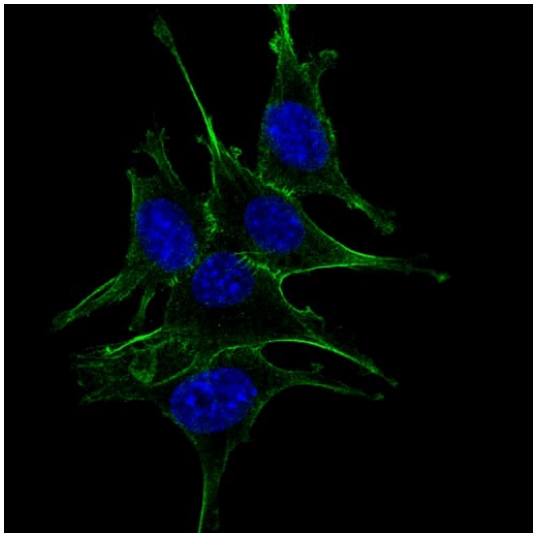
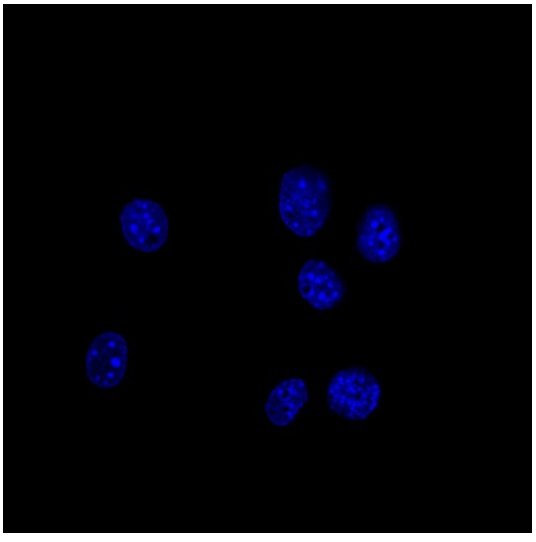


Antibody : CD31 P91 at 1/50 (BD Pharmingen)
Cell : NIH/3T3



Antibody : Isotype control
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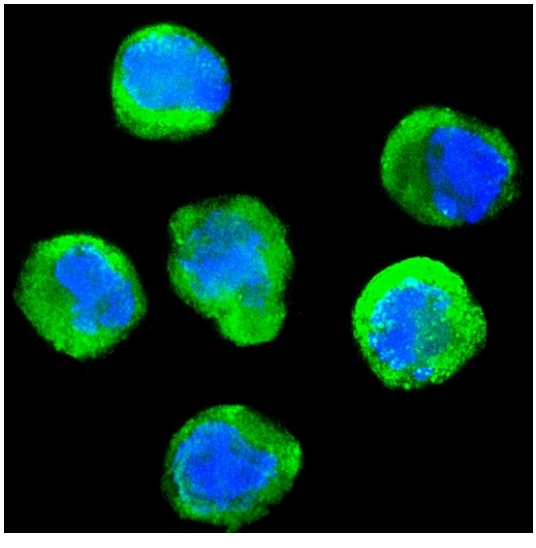

Antibody : CD31 P91 at 1/50 (BD Pharmingen)
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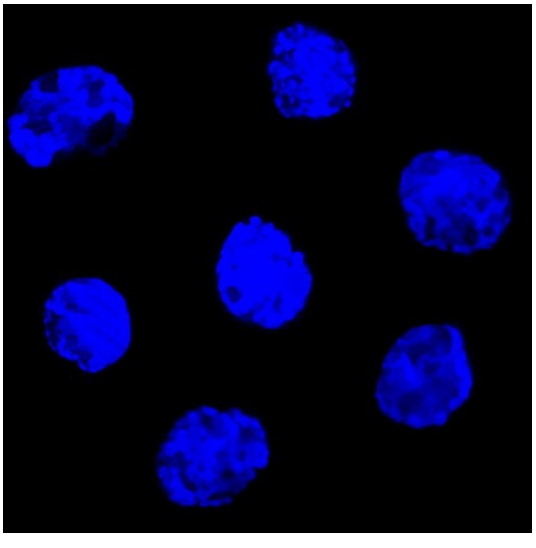

Antibody : Isotype control
Cell : THP-1

METHOD

Antibodies

	Primary antibody	Secondary antibody	Isotype Control
	CD31 M31 at 1/50 (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 1/50
	CD31 M32 at 1/50 (Supplier 06)	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 1/50
	CD31 M33 at 1/10 (Novus)	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 1/10
	CD31 M34 at 1/500 (Fitzgerald)	Goat anti-Rat IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, SA5-10018) at 1/200	Rat IgG2a Isotype Control (Thermo Scientific, PA5-33214) at 1/500
	CD31 P33 at 1/500 (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 1/500
	CD31 P34 at 1/500 (Supplier 11)	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 1/500
	CD31 P91 at 1/50 (BD Pharmingen)	Goat anti-Rat IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, SA5-10018) at 1/200	Rat IgG2a Isotype Control (Thermo Scientific, PA5-33214) at 1/50

= Component of the CD31 Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunofluorescent analysis of NIH/3T3 (Mouse embryo fibroblast) and THP-1 (Human monocytic leukemia) cells 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.

EXPERIMENTAL NOTES

The expected location of CD31 is on the cell membrane. Under these experimental conditions, CD31 M33 and CD31 M34 exhibit membrane staining on both the Mouse and Human derived cells. CD31 P91 shows membrane only staining of the NIH/3T3 cells but both membrane some cytoplasm staining on the THP-1 cells.

Membrane and some cytoplasm staining is also detected by CD31 M31 in both cell types whilst the other antibodies (CD31 M32, CD31 P33 and CD31 P34) demonstrate more non localized staining across the membrane, cytoplasm and nuclei of the cells.

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 $225, €196, £145.

The CD31 Superstarter Antibody Panel consists of:
-1x BD Biosciences 550274 (star performer)
-1x Abcam ab28364 (high ratings)
-1x Santa Cruz Biotechnology sc-1506 (high ratings)
http://www.antibodyresource.com/superstars

Images of Superstar CD31 antibodies: