

ANTIBODY TESTING REPORT

SUMMARY

Antigen: IL6 (Uniprot# P05231)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

Project ID: AR126

BACKGROUND

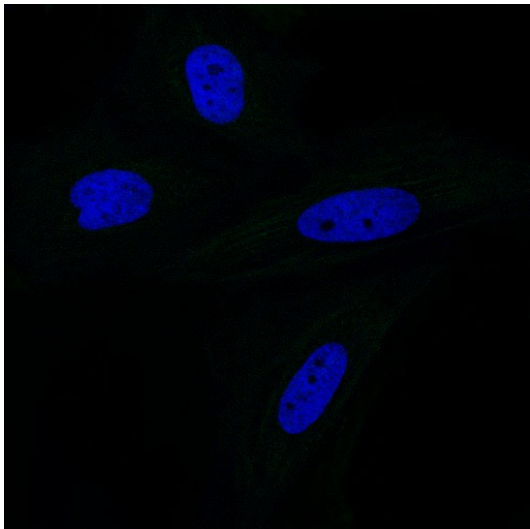
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical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

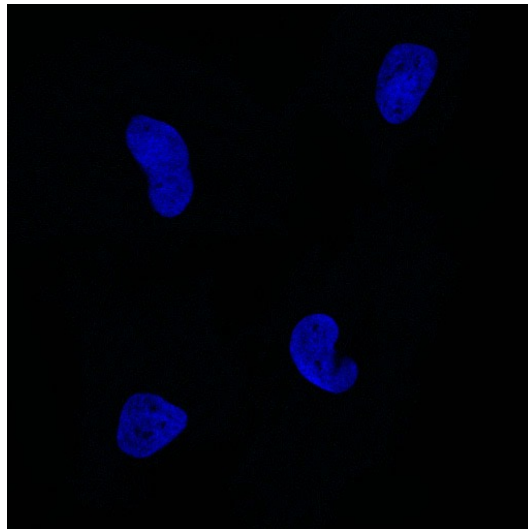
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

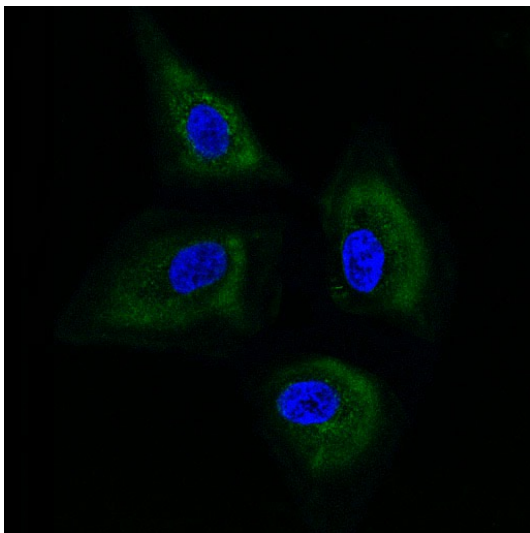
Immunofluorescence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-IL6 antibodies (green) and isotype controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



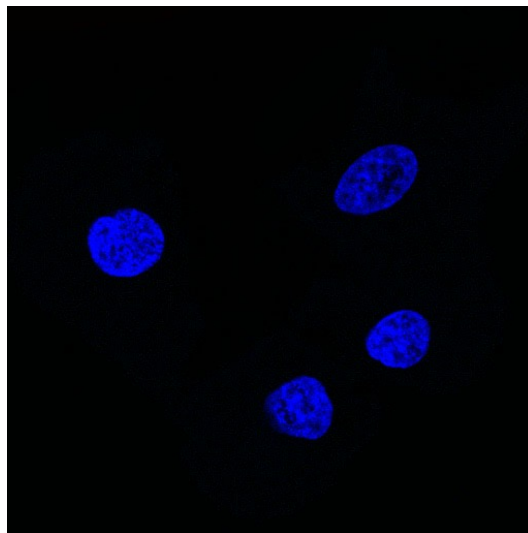
Antibody : IL6 P65 at 1/500 (St John's Lab)
Cell : HeLa



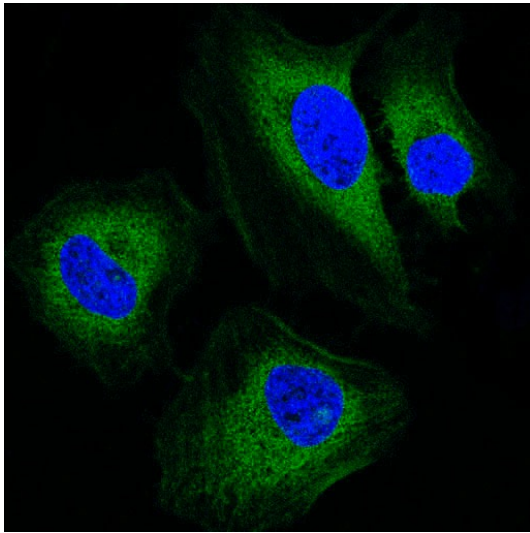
Antibody : Isotype control
Cell : HeLa



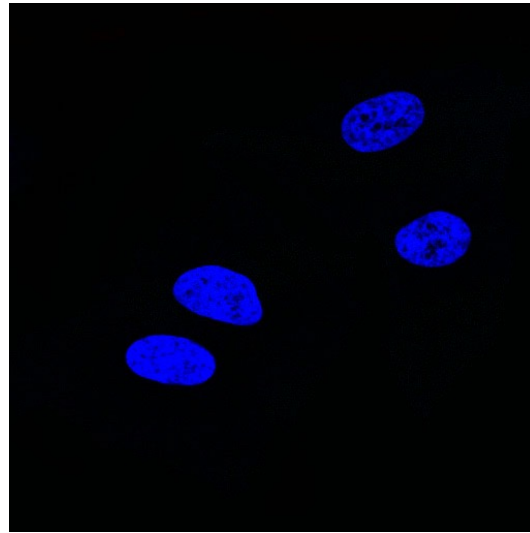
Antibody : IL6 P65 at 1/500 (St John's Lab)
Cell : A549



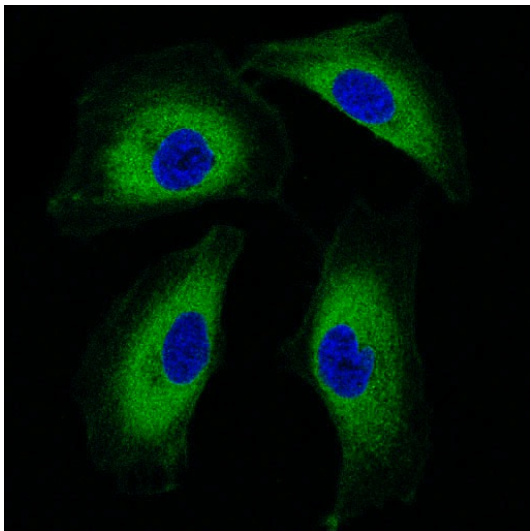
Antibody : Isotype control
Cell : A549



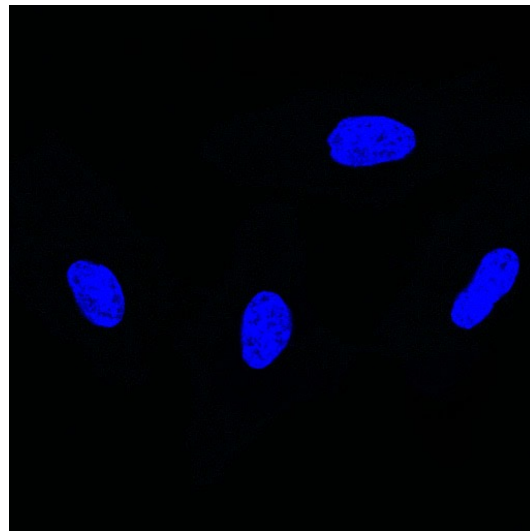
Antibody : [IL6 M75 at 1/500 \(Absea\)](#)
Cell : HeLa



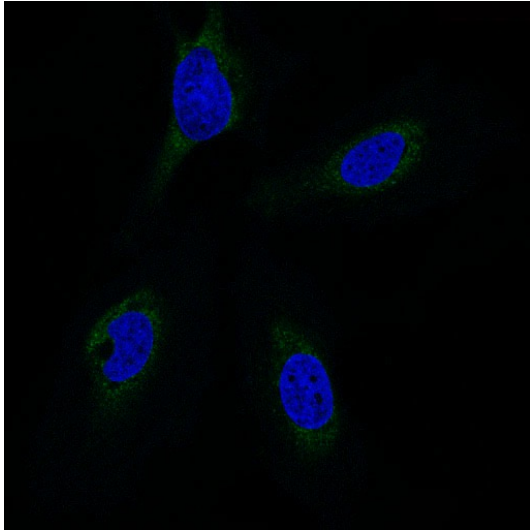
Antibody : Isotype control
Cell : HeLa



Antibody : [IL6 M75 at 1/500 \(Absea\)](#)
Cell : A549

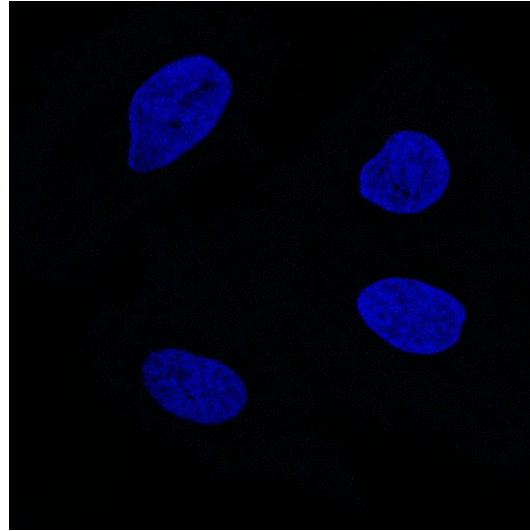


Antibody : Isotype control
Cell : A549



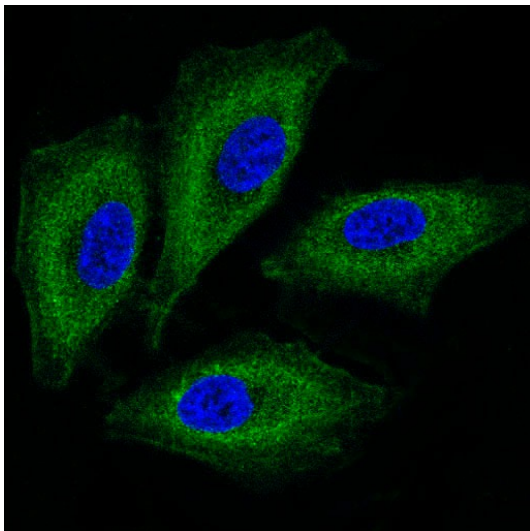
Antibody : [IL6 M76 at 1/500 \(Absea\)](#)

Cell : HeLa



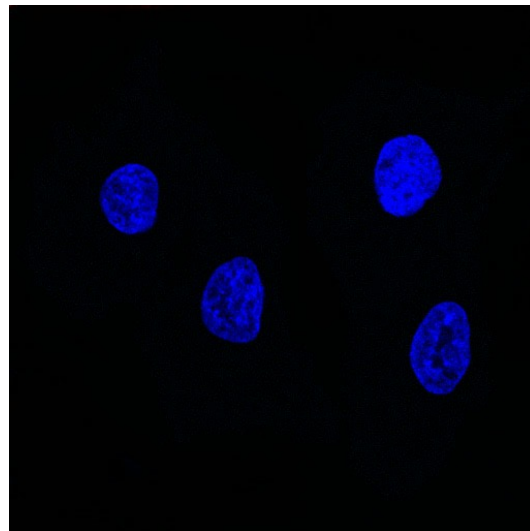
Antibody : Isotype control

Cell : HeLa



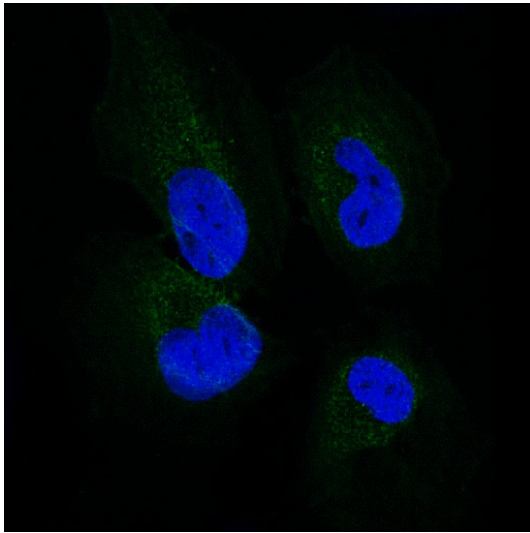
Antibody : [IL6 M76 at 1/500 \(Absea\)](#)

Cell : A549



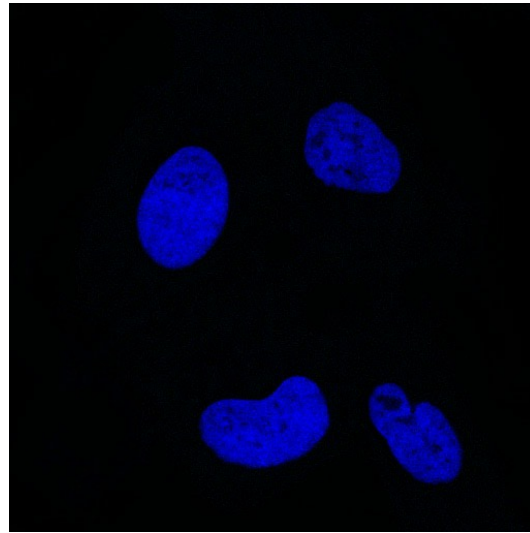
Antibody : Isotype control

Cell : A549



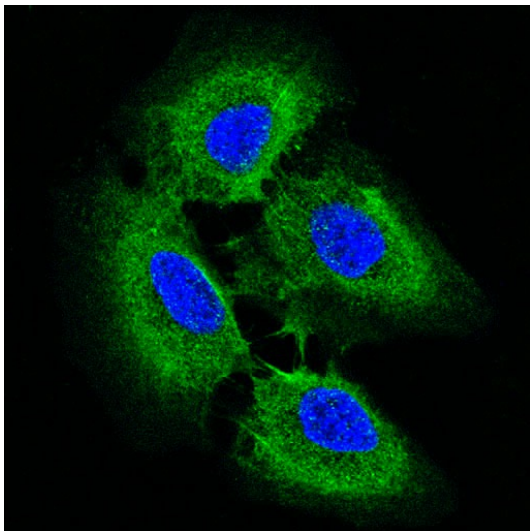
Antibody : [IL6 P66 at 1/500 \(Boster\)](#)

Cell : HeLa



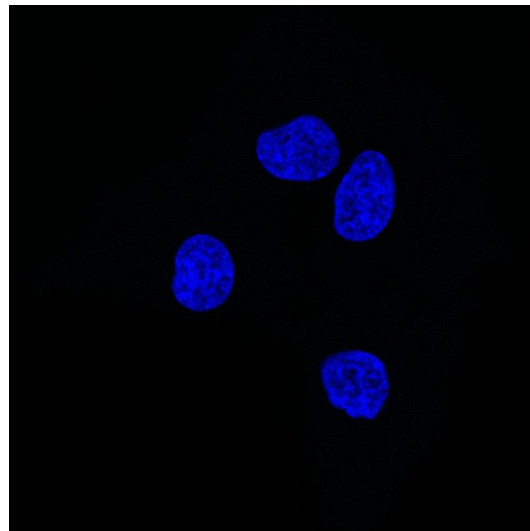
Antibody : Isotype control

Cell : HeLa



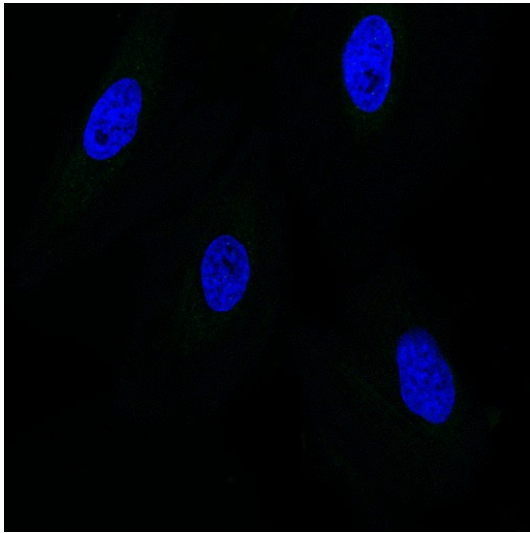
Antibody : [IL6 P66 at 1/500 \(Boster\)](#)

Cell : A549

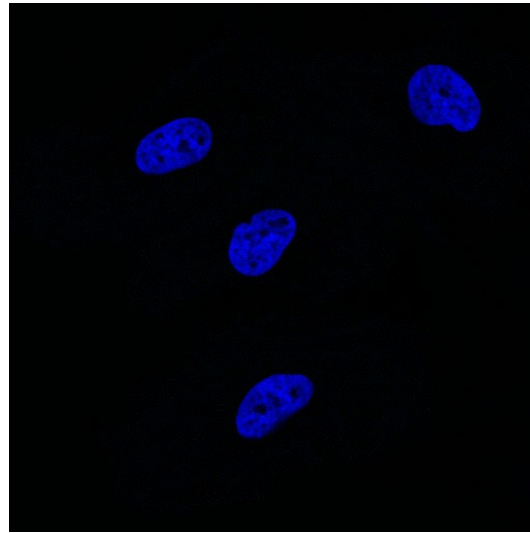


Antibody : Isotype control

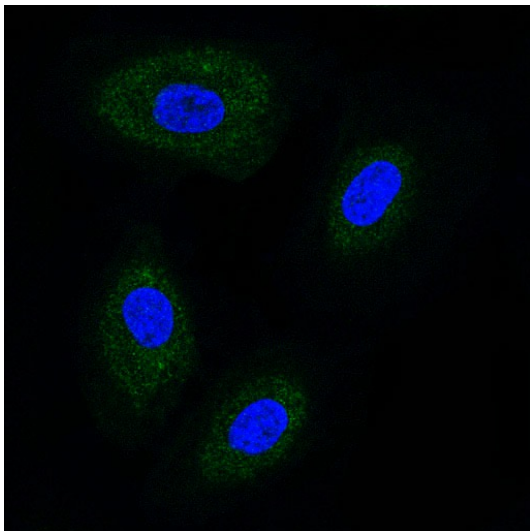
Cell : A549



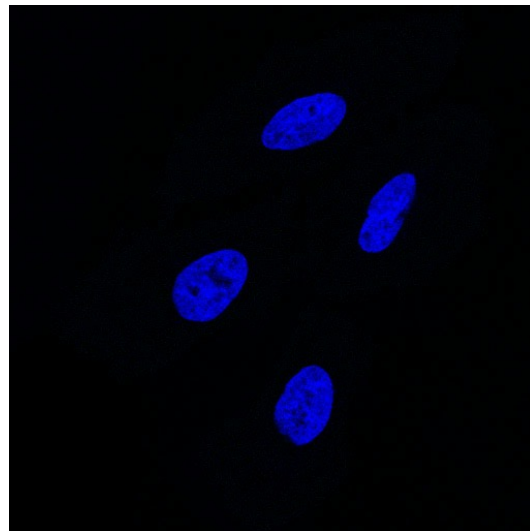
Antibody : [IL6 M77 at 1/500 \(Fitzgerald\)](#)
Cell : HeLa



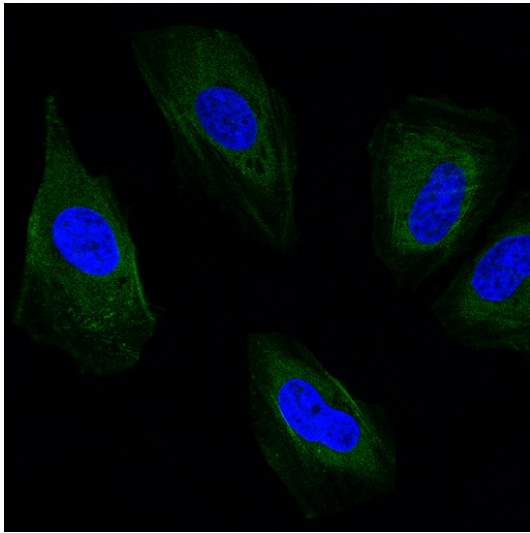
Antibody : Isotype control
Cell : HeLa



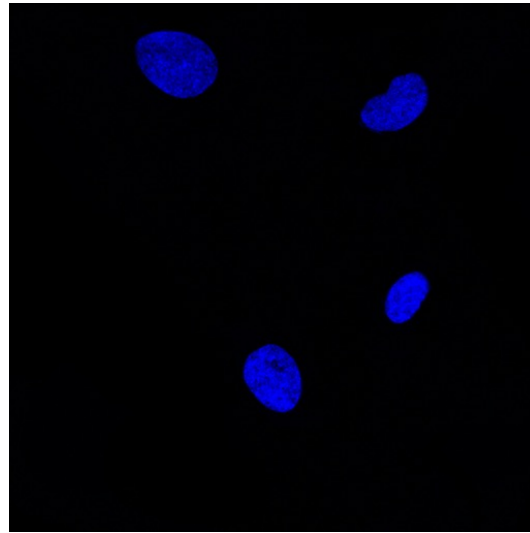
Antibody : [IL6 M77 at 1/500 \(Fitzgerald\)](#)
Cell : A549



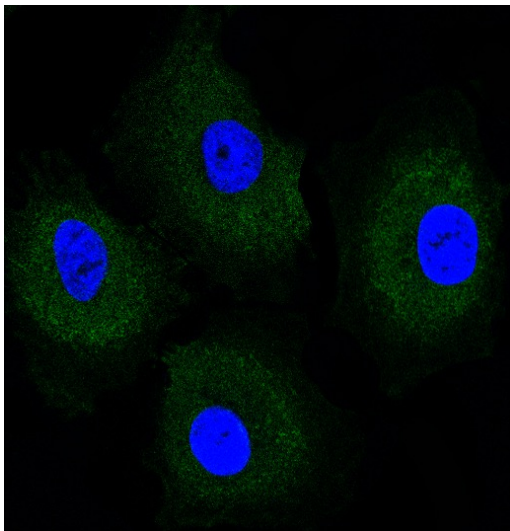
Antibody : Isotype control
Cell : A549



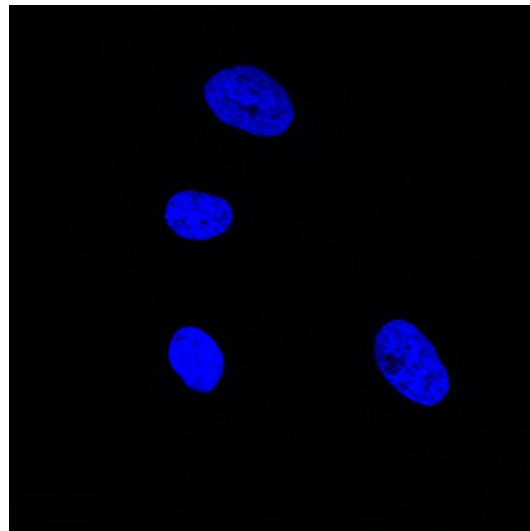
Antibody : [IL6 P89 at 1/50 \(R & D Systems\)](#)
Cell : HeLa



Antibody : Isotype control
Cell : HeLa




Antibody : [IL6 P89 at 1/50 \(R & D Systems\)](#)
Cell : A549



Antibody : Isotype control
Cell : A549

METHOD

Antibodies

	Primary antibody	Secondary antibody	Isotype Control
	IL6 P65 at 1/500 (St John's Laboratory)	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
	<u>IL6 M75 at 1/500</u> <u>(Absea)</u>	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 1/500
	<u>IL6 M76 at 1/500</u> <u>(Absea)</u>	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG2b Isotype Control (Thermo Scientific, MA5-14447) at 1/500
	<u>IL6 P66 at 1/500</u> <u>(Boster)</u>	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
	<u>IL6 M77 at 1/500</u> <u>(Fitzgerald)</u>	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
	<u>IL6 P89 at 1/50</u> <u>(R & D Systems)</u>	Alexa Fluor® 488 AffiniPure Donkey Anti-Goat IgG(H+L) (Jackson ImmunoResearch, 805-545-180) at 1/200	Goat IgG Isotype Control (Life Technologies, 02-6202) at 1/50

 = Component of the IL6 Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunofluorescent analysis of HeLa (Human epithelial cells from cervix adenocarcinoma) cells and A549 (Human lung carcinoma) 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°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

IL6 is a secreted protein and would be expected to show cytoplasm staining by immunofluorescent analysis according to Uniprot# P05231. Under these experimental conditions, IL6 M75 exhibits localized cytoplasm staining in both cell lines whilst IL6 M76 and IL6 P66 show cytoplasm staining of the A549 cells only. The HeLa binding may require confirmation at a higher concentration of the antibodies. This may also be true for the binding of IL6 P65, IL6 P89 and IL6 M77 as only weak cytoplasm staining is suggested using these antibodies.

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 \$287, €267, £198.

The IL6 Superstarter Antibody Panel consists of:

- 1x [ab9770](#) Abcam (high ratings)
 - 1x [MAB406](#) R&D Systems (high ratings)
 - 1x [sc-1265](#) Santa Cruz Biotechnology (star performer)
- <http://www.antibodyresource.com/superstars>

Images of Superstar IL6 antibodies:

