

ANTIBODY TESTING REPORT

SUMMARY

Antigen: GFAP (Uniprot# P03995)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

Project ID: AR125

BACKGROUND

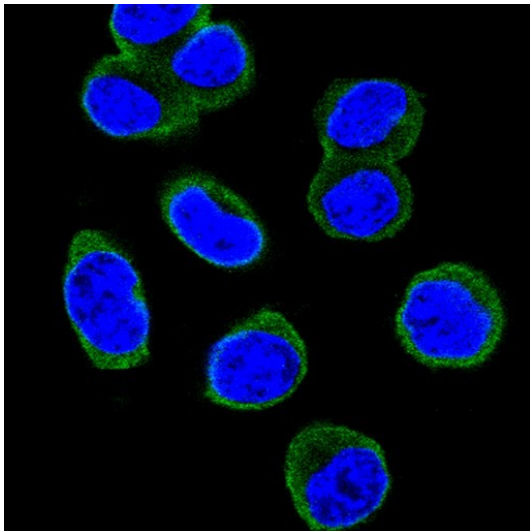
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical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

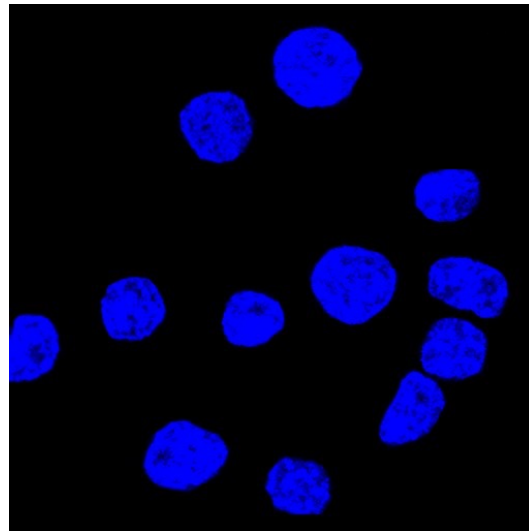
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

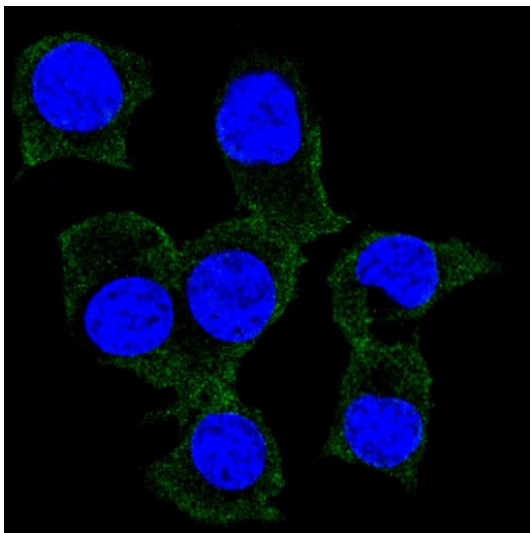
Immunofluorescence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-GFAP antibodies (green) and isotype controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



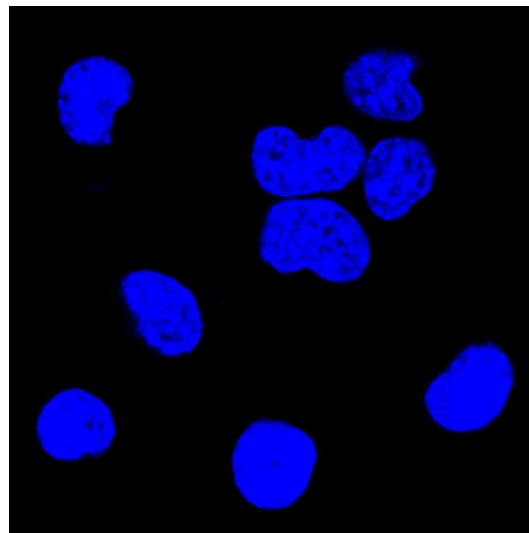
Antibody : GFAP P15 at 1/1000 (Supplier 08)
Cell : Neuro-2a



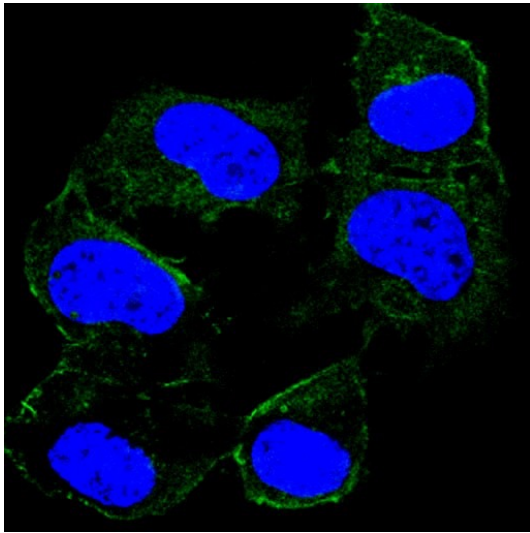
Antibody : Isotype control
Cell : Neuro-2a



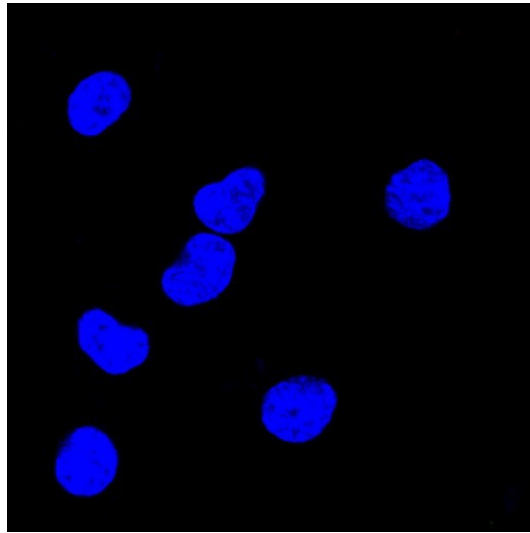
Antibody : GFAP M12 at 1/1000 (Supplier 29)
Cell : Neuro-2a



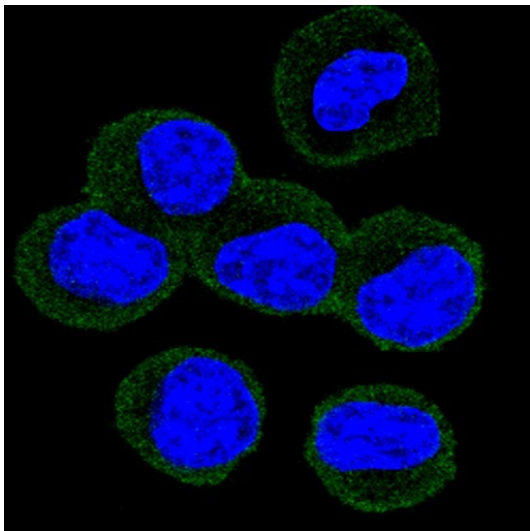
Antibody : Isotype control
Cell : Neuro-2a



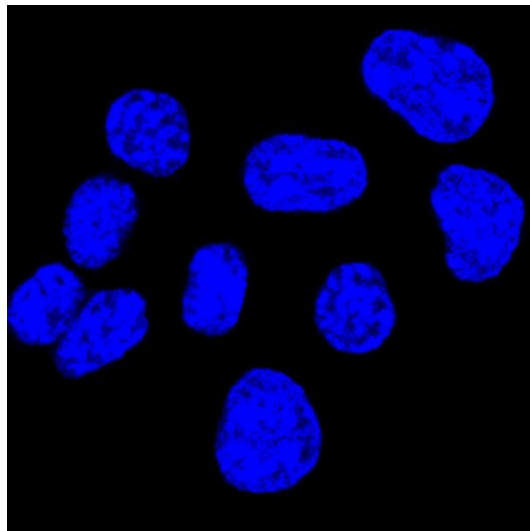
Antibody : [GFAP P16 at 1/1000 \(Atlas\)](#)
Cell : Neuro-2a



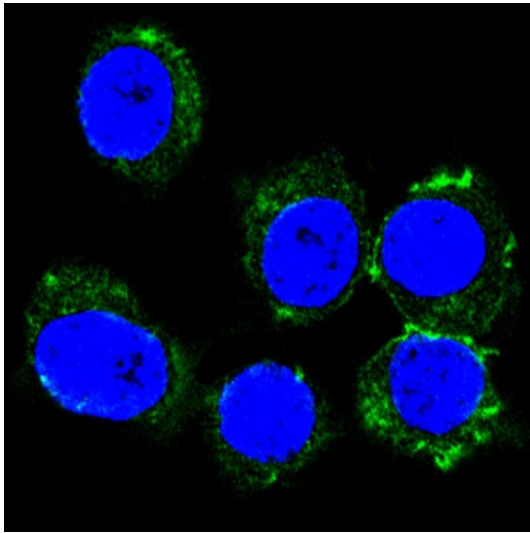
Antibody : Isotype control
Cell : Neuro-2a



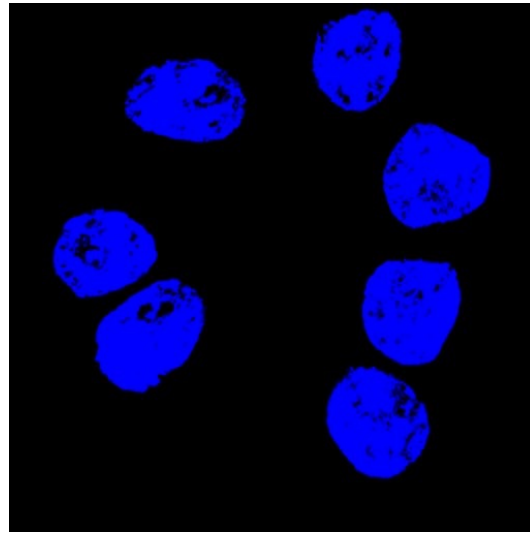
Antibody : GFAP P17 at 1/1000 (Supplier 06)
Cell : Neuro-2a



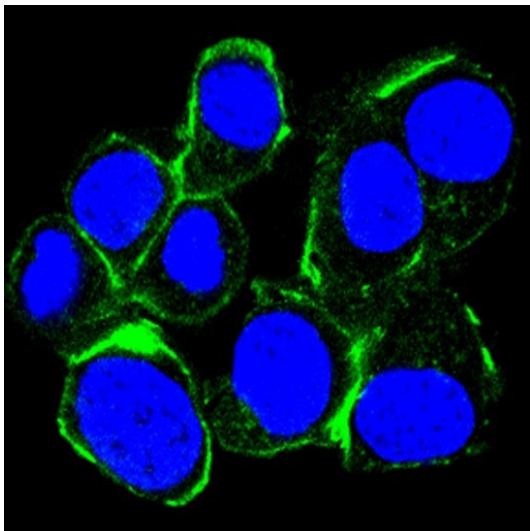
Antibody : Isotype control
Cell : Neuro-2a



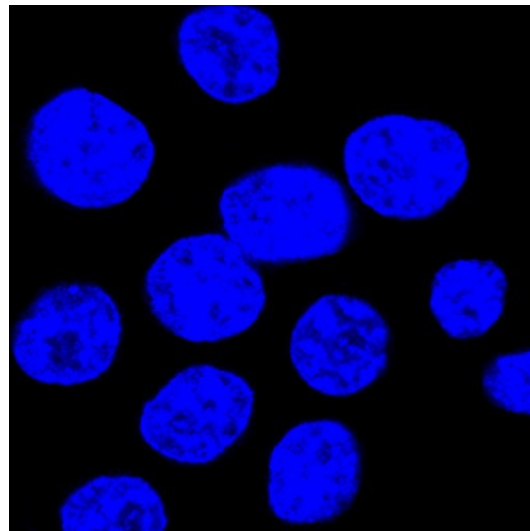
Antibody : [GFAP P18 at 1/1000 \(Novus\)](#)
Cell : Neuro-2a



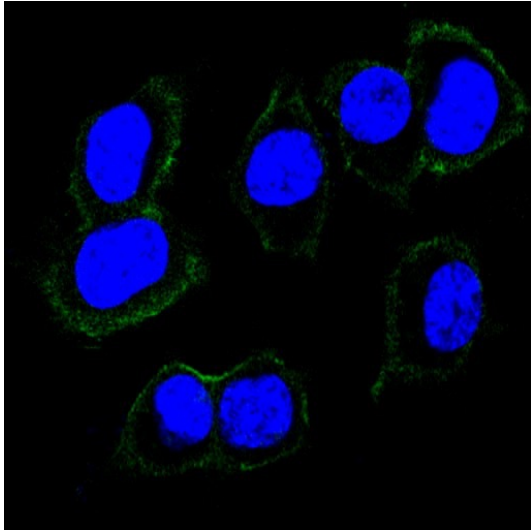
Antibody : Isotype control
Cell : Neuro-2a



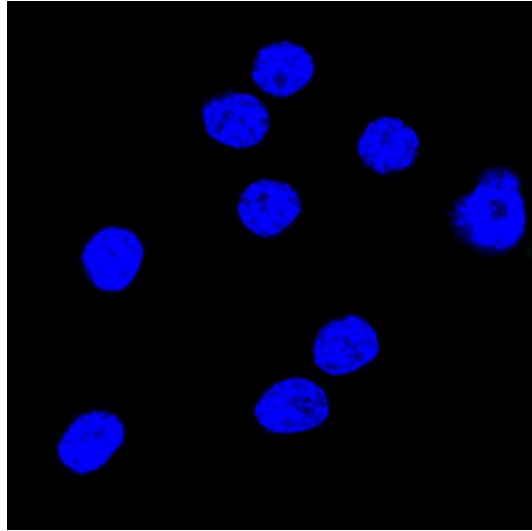
Antibody : [GFAP P26 at 1/1000 \(Boster\)](#)
Cell : Neuro-2a



Antibody : Isotype control
Cell : Neuro-2a



Antibody : GFAP M28 at 1/1000 (Supplier 16)
Cell : Neuro-2a



Antibody : Isotype control
Cell : Neuro-2a

METHOD

Antibodies

Primary antibody	Secondary antibody	Isotype Control
GFAP P15 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
GFAP M12 at 1/100 (Supplier 29)	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 1/100
GFAP P16 at 1/1000 (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 1/1000
GFAP P17 at 1/100 (Supplier 06)	Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG(H+L) (Jackson ImmunoResearch, 706-546-148) at 1/200	PBS
GFAP P18 at 1/1000 (Novus)	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 1/1000
GFAP P26 at 1/1000 (Boster)	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 1/1000
GFAP M28 at 1/100 (Supplier 16)	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

PROTOCOL

Immunofluorescent analysis of Neuro-2a cells (Mouse neuroblastoma) 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

The subcellular location of GFAP is within the cytoplasm, associated with intermediate filaments (Uniprot # P03995). Under these experimental conditions, GFAP P16, GFAP P18 and GFAP P26 exhibit staining consistent with this cellular location. The immunofluorescent staining using GFAP M12, GFAP M28, GFAP P15 and GFAP P17 suggests intermediate filament binding on the Neuro-2a cells but requires confirmation as the binding could be interpreted as cytoplasm only 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 \$318, €277, £206.

The GFAP Superstarter Antibody Panel consists of:

- 1x Abcam [ab7260](#) (high ratings)
 - 1x Millipore [MAB360](#) (high ratings)
 - 1x Sigma-Aldrich [G3893](#) (star performer)
- <http://www.antibodyresource.com/superstars>

Images of Superstar GFAP antibodies:

