ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: TAU (Uniprot# P10636)

Method tested: Immunofluorescent staining of cells

Laboratory ID: LAB07

Project ID: AR127

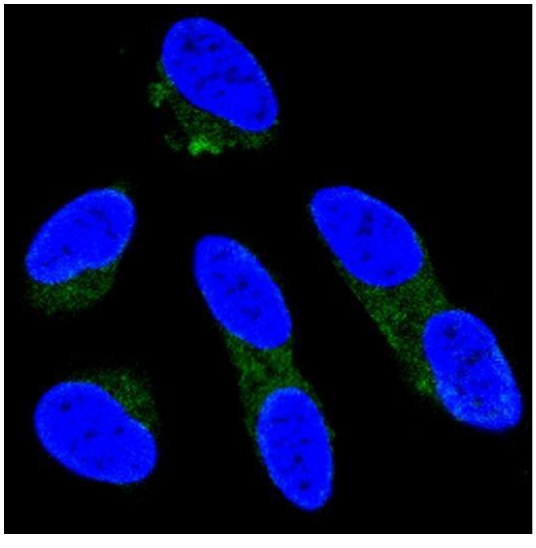
BACKGROUND

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.

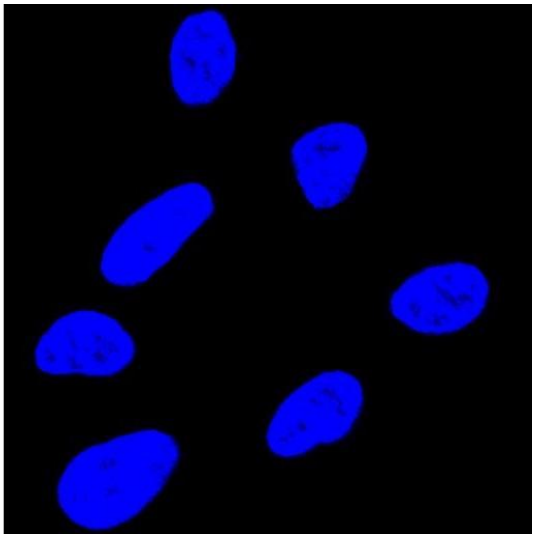
For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID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

RESULTS

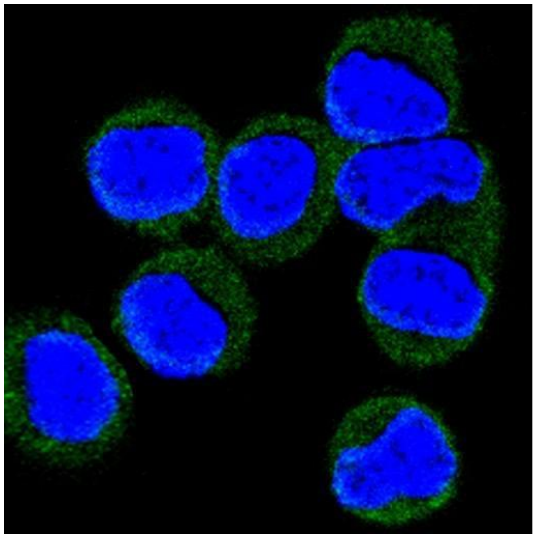
Immunofluoresence analysis of formalin fixed, 0.1% Triton X-100 permeabilized cells using various anti-TAU antibodies (green) and controls (see Method section for more detail). The nuclear counter stain is DAPI (blue).



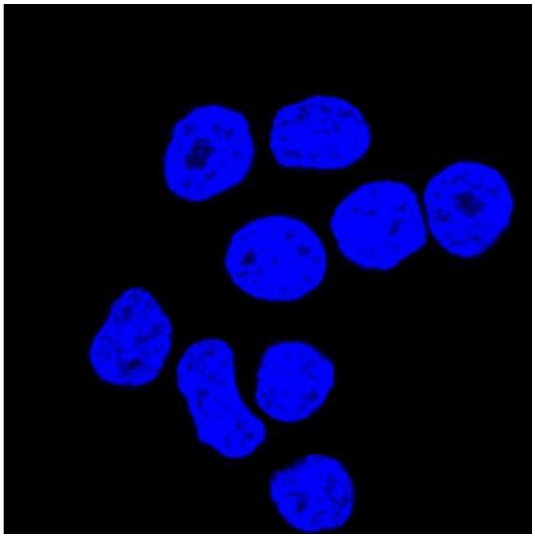
Antibody : TAU P76 at 1/1000 (Supplier 08)
Cell : SH-SY5Y



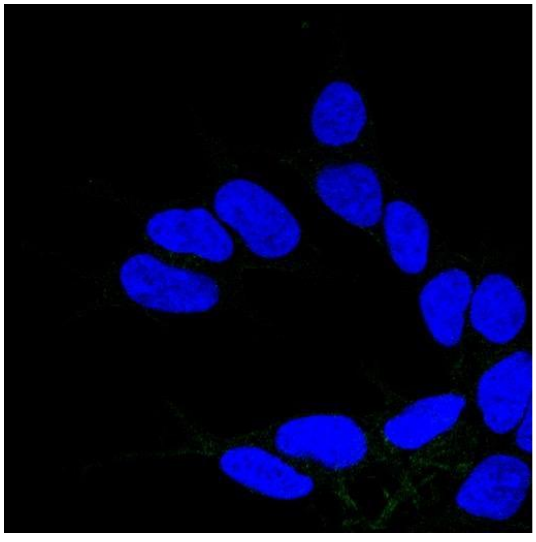
Antibody : Negative control
Cell : SH-SY5Y



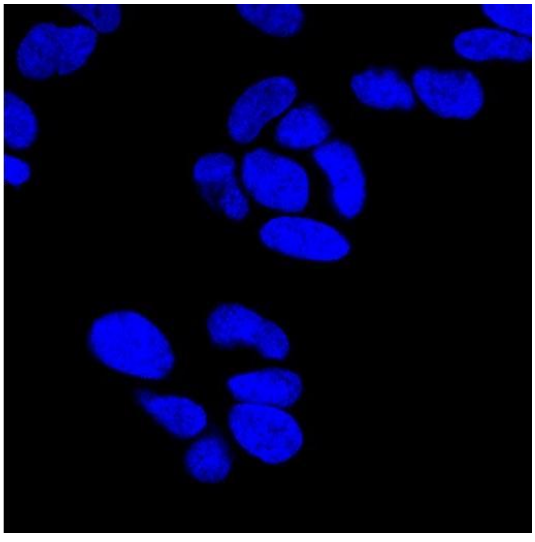
Antibody : TAU P76 at 1/1000 (Supplier 08)
Cell : Neuro-2a



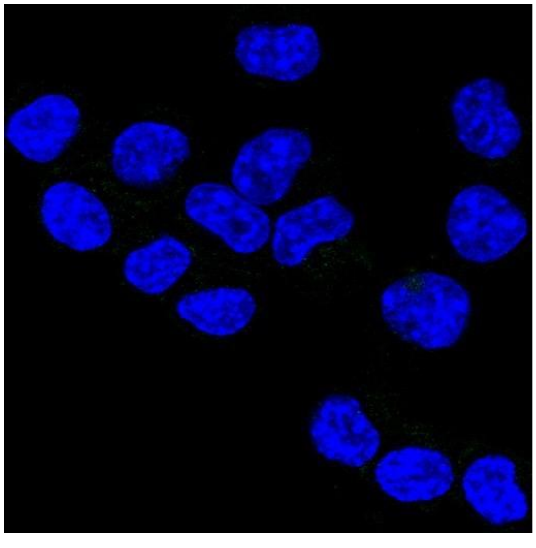
Antibody : Negative control
Cell : Neuro-2a



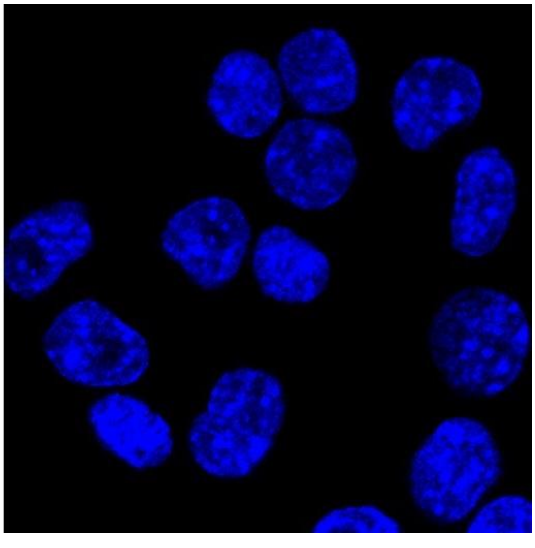
Antibody : TAU P78 at 1/1000 (Supplier 11)
Cell : SH-SY5Y



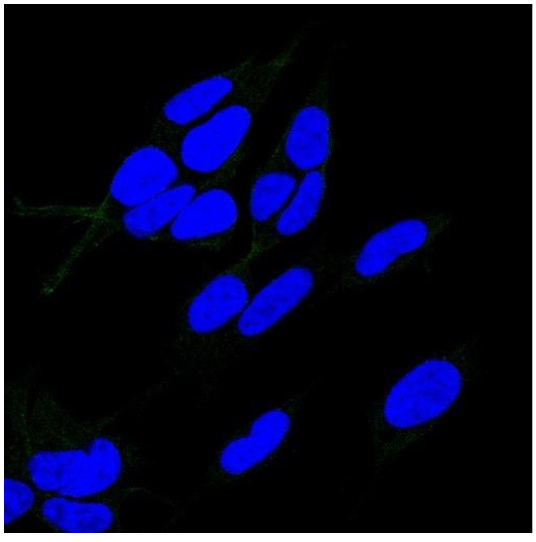
Antibody : Isotype control
Cell : SH-SY5Y



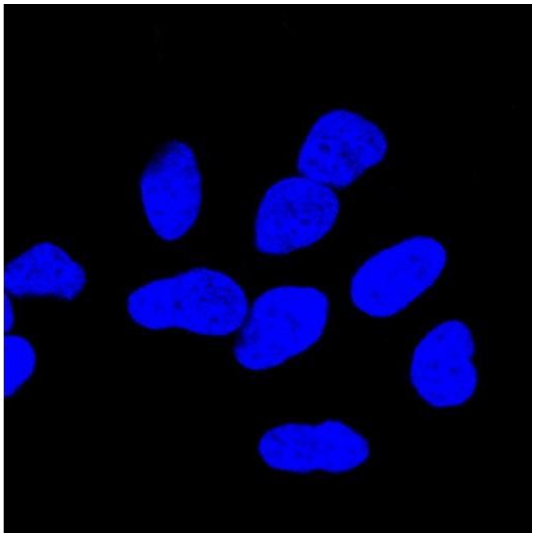
Antibody : TAU P78 at 1/1000 (Supplier 11)
Cell : Neuro-2a



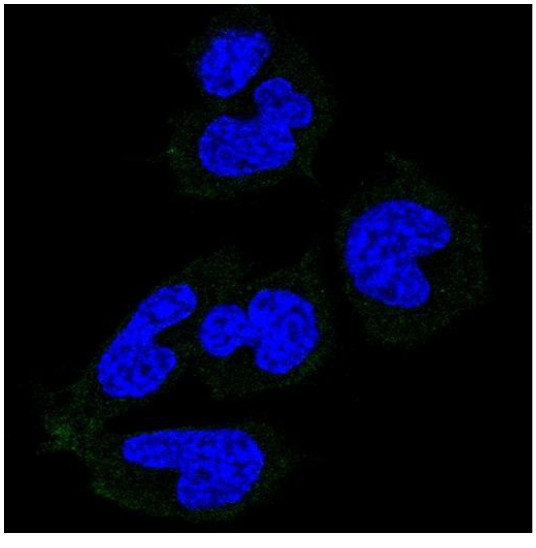
Antibody : Isotype control
Cell : Neuro-2a



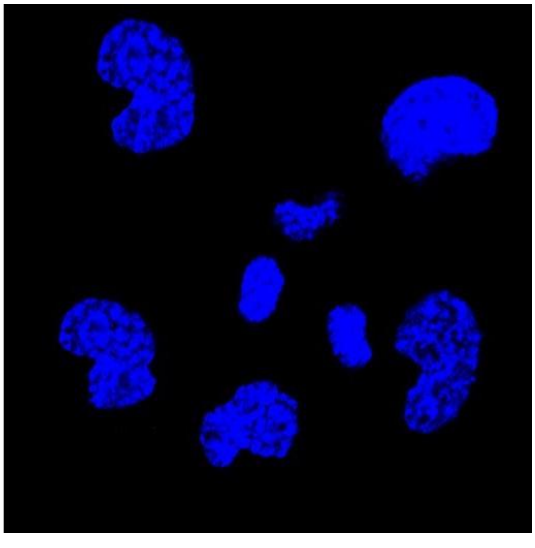
Antibody : TAU M84 at 1/1000 (Supplier 16)
Cell : SH-SY5Y



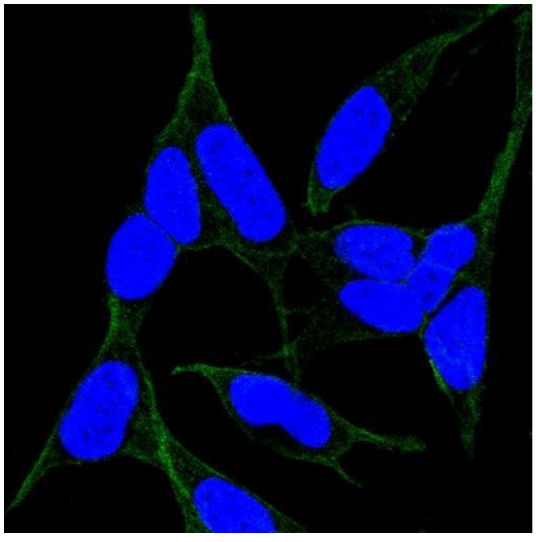
Antibody : Isotype control
Cell : SH-SY5Y



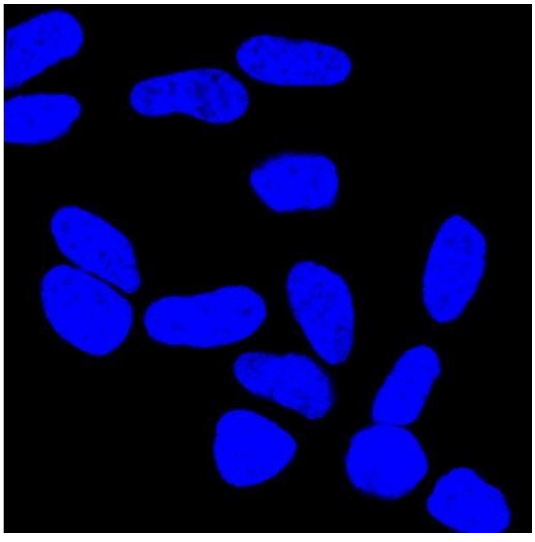
Antibody : TAU M84 at 1/1000 (Supplier 16)
Cell : Neuro-2a



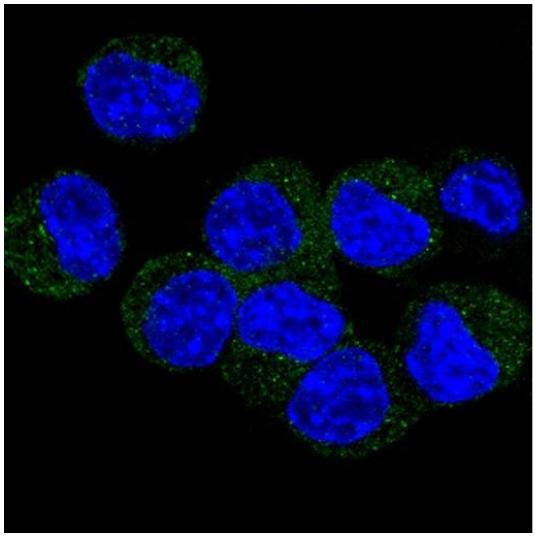
Antibody : Isotype control
Cell : Neuro-2a



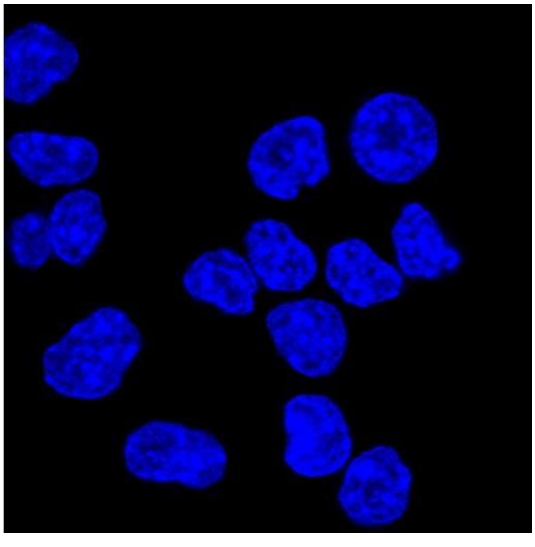
Antibody : TAU M85 at 1/1000 (Synaptic Systems)
Cell : SH-SY5Y



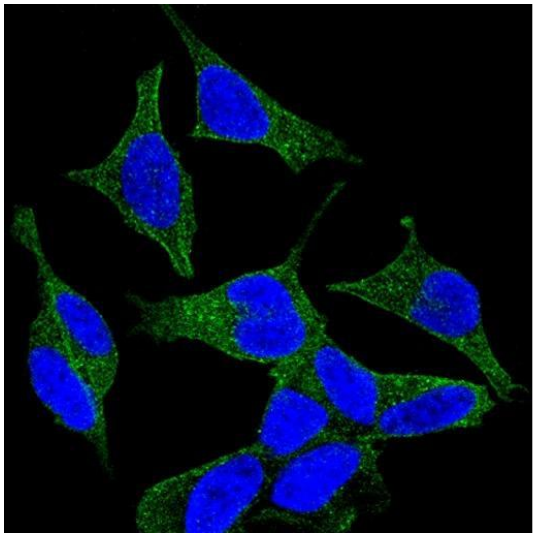
Antibody : Isotype control
Cell : SH-SY5Y



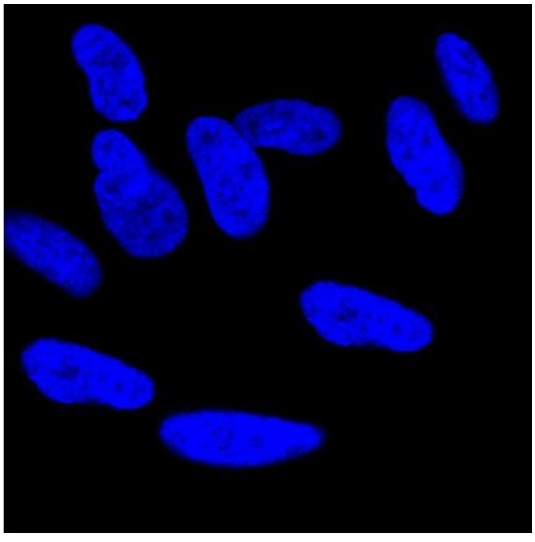
Antibody : TAU M85 at 1/1000 (Synaptic Systems)
Cell : Neuro-2a



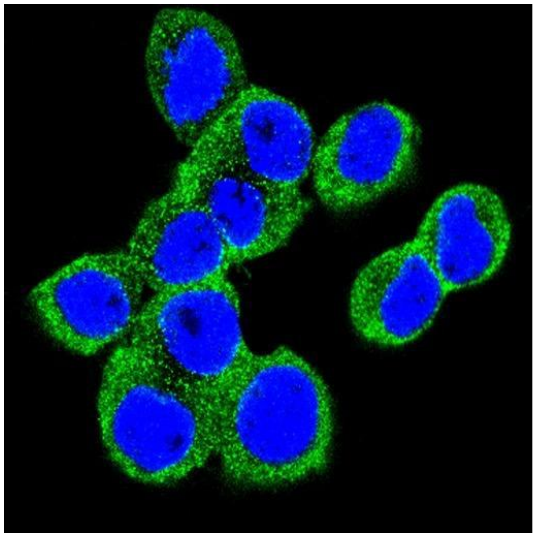
Antibody : Isotype control
Cell : Neuro-2a



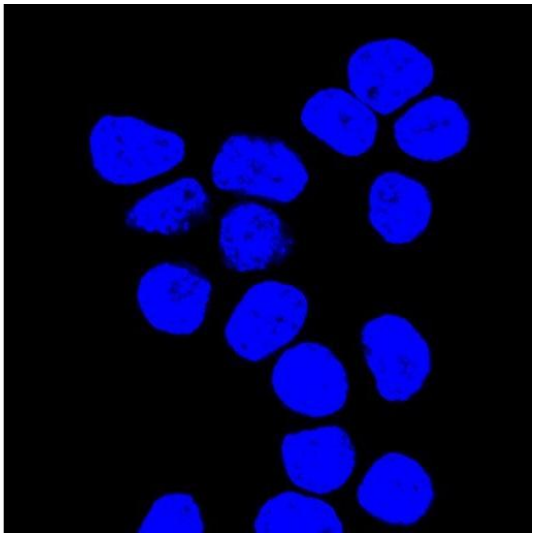
Antibody : TAU P79 at 1/1000 Synaptic Systems)
Cell : SH-SY5Y



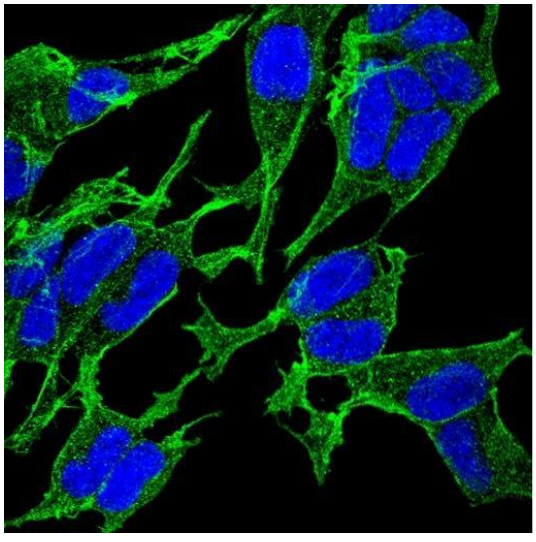
Antibody : Isotype control
Cell : SH-SY5Y



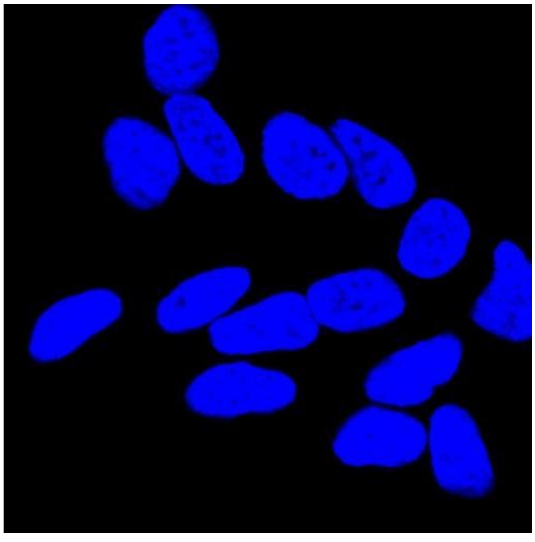
Antibody : TAU P79 at 1/1000 Synaptic Systems)
Cell : Neuro-2a



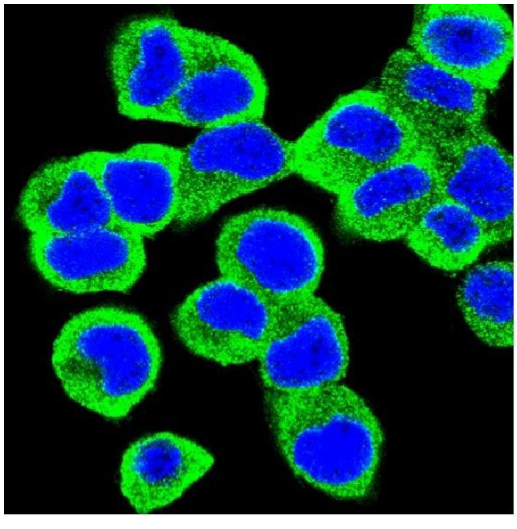
Antibody : Isotype control
Cell : Neuro-2a



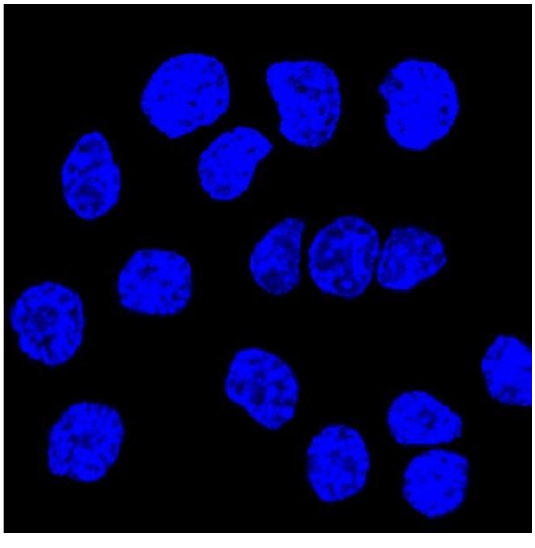
Antibody : TAU P80 at 1/1000 Synaptic Systems)
Cell : SH-SY5Y



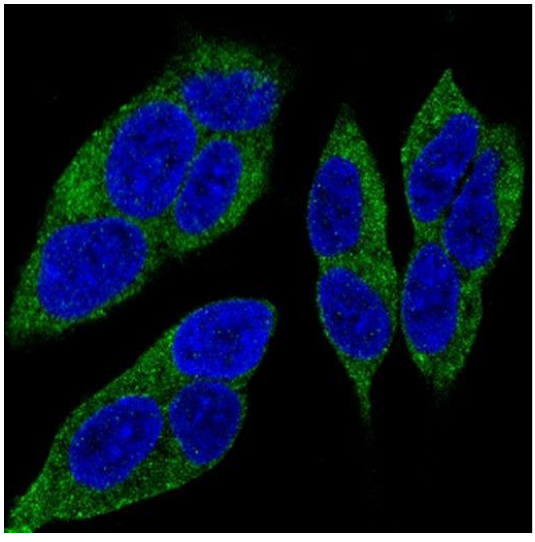
Antibody : Isotype control
Cell : SH-SY5Y



Antibody : TAU P80 at 1/1000 Synaptic Systems)
Cell : Neuro-2a

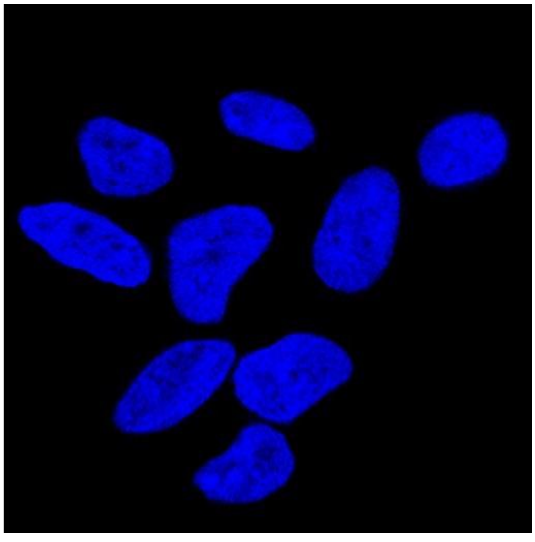


Antibody : Isotype control
Cell : Neuro-2a



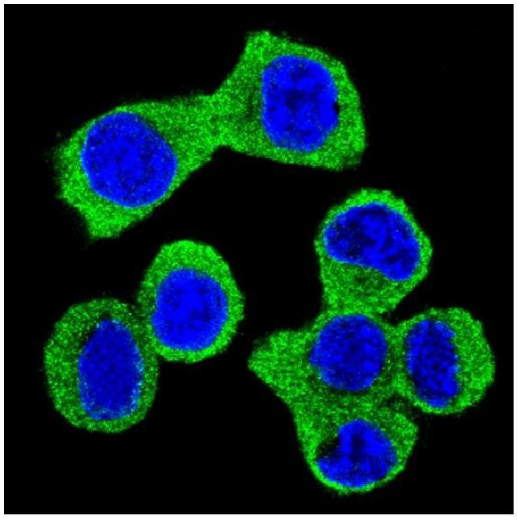
Antibody : TAU P81 at 1/1000 (Synaptic Systems)

Cell : SH-SY5Y



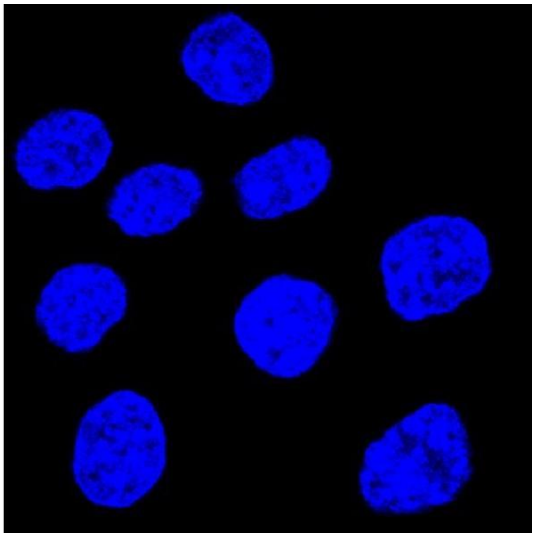
Antibody : Negative control

Cell : SH-SY5Y



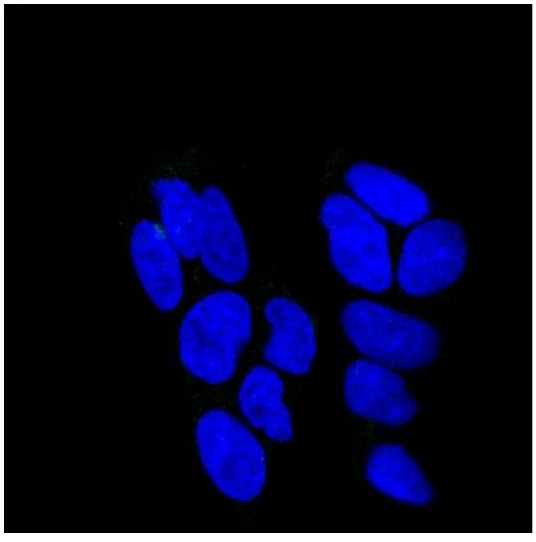
Antibody : TAU P81 at 1/1000 (Synaptic Systems)

Cell : Neuro-2a

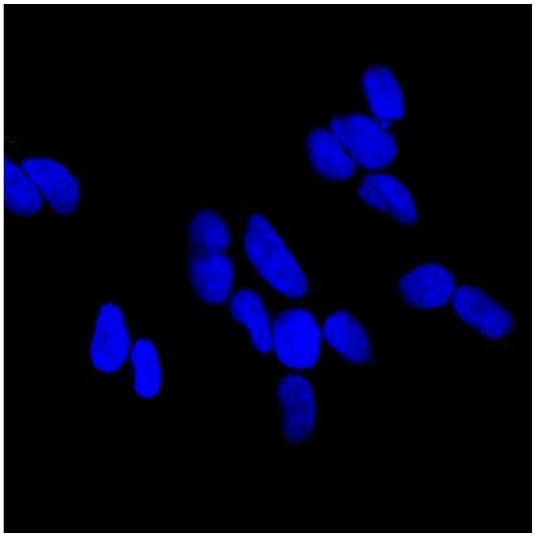


Antibody : Negative control

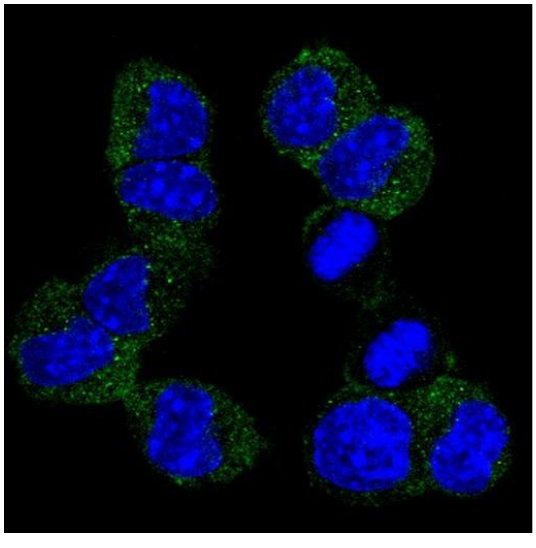
Cell : Neuro-2a



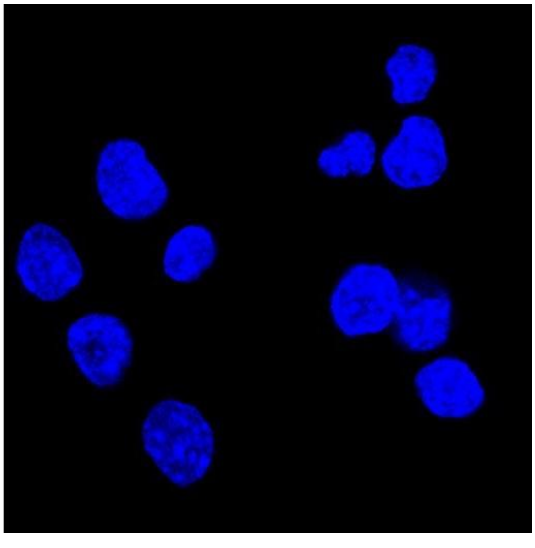
Antibody : TAU M92 at 1/1000 (Millipore)
Cell : SH-SY5Y



Antibody : Isotype control
Cell : SH-SY5Y



Antibody : TAU M92 at 1/1000 (Millipore)
Cell : Neuro-2a



Antibody : Isotype control
Cell : Neuro-2a

METHOD

Antibodies

	Primary antibody	Secondary antibody	Control
	TAU P76 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
	TAU P78 at 1/1000 (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 1/1000
	TAU M84 at 1/1000 (Supplier 16)	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 1/1000
	TAU M85 at 1/1000 (Synaptic Systems)	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 1/1000
	TAU P79 at 1/1000 (Synaptic Systems)	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
	TAU P80 at 1/1000 (Synaptic Systems)	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
	TAU P81 at 1/1000 (Synaptic Systems)	Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L) (Jackson ImmunoResearch, 706-545-148) at 1/200	PBS
	TAU M92 at 1/1000 (Millipore)	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 1/1000

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

PROTOCOL

Immunofluorescent analysis of SH-SY5Y cells (Human neuroblastoma from bone marrow cells) and Neuro-2a cells (Mouse neuroblastoma 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

Under these experimental conditions, TAU P79, TAU P80 and TAU P81 exhibit both membrane and cytoplasm staining on both the Human and Mouse cells whilst TAU M92 shows staining of the Mouse cells only. TAU M85 indicates binding to the membrane of the Human cells and the cytoplasm of the Mouse cells; TAU P76 demonstrates staining of the cytoplasm only for both cell species. Neither TAU P78 nor TAU M84 showed any cell binding in this experiment. The TAU M84, TAU M85, TAU P76 and TAU P78 staining patterns may require confirmation at a higher concentrations of the 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 $307, €267, £198.

The TAU Superstarter Antibody Panel consists of:

-1x Millipore MAB3420
-1x Santa Cruz Biotechnology sc-5587
-1x Abcam ab64193
-1x Novus NBP2-25162
-1x Aves TAU

http://www.antibodyresource.com/superstars

Images of Superstar TAU antibodies: