ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: TAU (Uniprot# P10636)

Method tested: Western Blotting

Laboratory ID: LAB07

Project ID: AR120

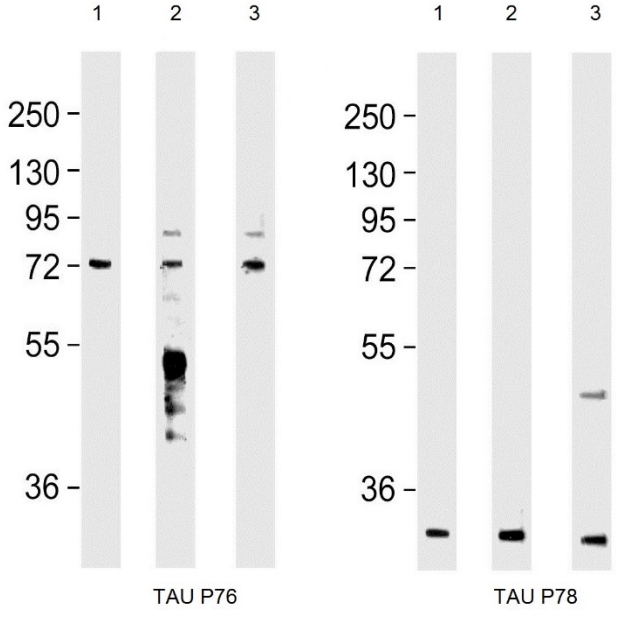
BACKGROUND

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



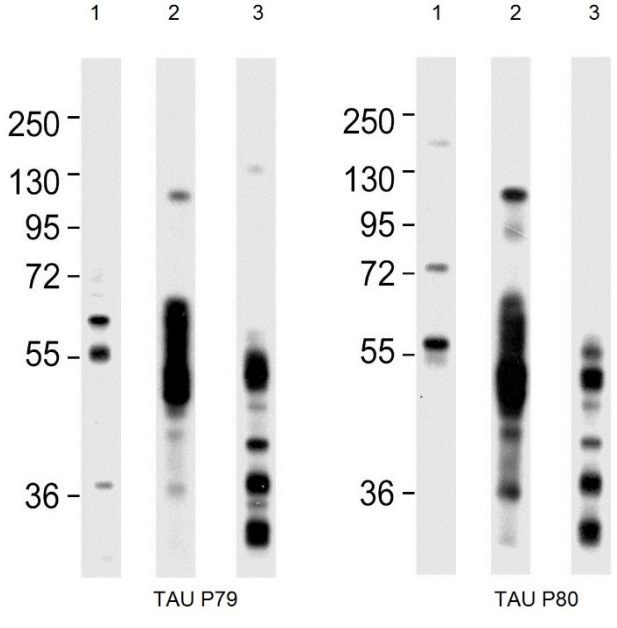
Western blot analysis of:-

(1) SH-SY5Y whole cell lysate

(2) Mouse brain tissue lysate

(3) Human brain tissue lysate

using TAU P76 and TAU P78 (see Method for primary and secondary antibody details).



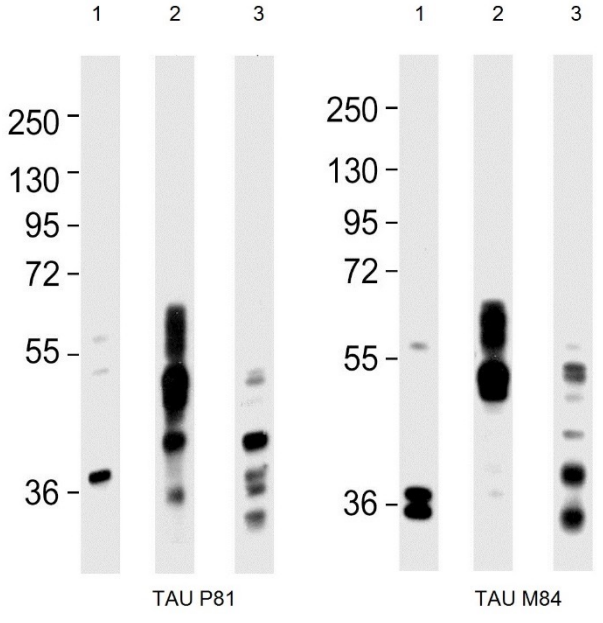
Western blot analysis of:-

(1) SH-SY5Y whole cell lysate

(2) Mouse brain tissue lysate

(3) Human brain tissue lysate

using TAU P79 and TAU P80 (see Method for primary and secondary antibody details).



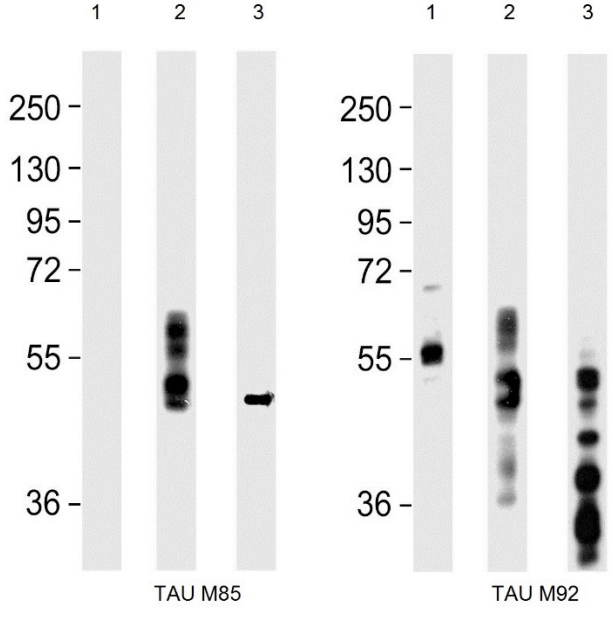
Western blot analysis of:-

(1) SH-SY5Y whole cell lysate

(2) Mouse brain tissue lysate

(3) Human brain tissue lysate

using TAU P81 and TAU M82 (see Method for primary and secondary antibody details).



Western blot analysis of:-

(1) SH-SY5Y whole cell lysate

(2) Mouse brain tissue lysate

(3) Human brain tissue lysate

using TAU M85 and TAU M92 (see Method for primary and secondary antibody details).

METHOD

Antibodies

Primary antibody	Secondary antibody
TAU P76 at 1/1000 (Aves)	Peroxidase AffiniPure Goat Anti-Chicken IgY (IgG) (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 103-035-155) at 1/10,000
TAU P78 at 1/1000 (Supplier 11)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
TAU P79 at 1/1000 (Supplier 30)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
TAU P80 at 1/1000 (Synaptic Systems)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
TAU P81 at 1/1000 (Supplier 30)	Peroxidase AffiniPure Goat Anti-Guinea Pig IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 106-035-003) at 1/10,000
TAU M84 at 1/1000 (Supplier 16)	Anti-Mouse IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, Cat no. A0168) at 1/10,000
TAU M85 at 1/1000 (Supplier 30)	Anti-Mouse IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, Cat no. A0168) at 1/10,000
TAU M92 at 1/1000 (Millipore)	Anti-Mouse IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, Cat no. A0168) at 1/10,000

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
SH-SY5Y (Human epithelial cells of neuroblastoma from bone marrow cells) whole cell lysate at 20 µg/lane	Lane 1 - Test
Mouse brain tissue lysate at 20 µg/lane	Lane 2 - Test
Human brain tissue lysate at 20 µg/lane	Lane 3 – Test

Detection Kit

Clarity™ Western ECL Blotting Substrate (Bio-rad, Cat no: 170-5061, Lot number: 102030607).

PROTOCOL

Western Blotting was performed using Invitrogen's Novex® XCell SureLock® Mini-Cell electrophoresis system followed by semi dry transfer onto PVDF membranes using Bio-Rad's Trans-Blot® SD Semi-Dry Transfer Cell and visualized using X-ray film as follows:-

1. Samples (see table above) were incubated with 1X SDS Sample Buffer containing 2% SDS and 100mM DTT at 95^{o}C for 5 minutes prior to loading.
2. The samples were then loaded and resolved on a 10% SDS-polyacrylamide gel (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by semi dry transfer and confirmed by amido black staining.
4. The immunoblot membrane was blocked in Tris buffered saline (TBS) containing Tween-20 (TBST) and 5% non-fat dry milk powder (blocking buffer) for 2 hours at room temperature with gentle agitation and then washed for 5 minutes in TBST.
5. The membrane was then immersed with the protein side up in the primary antibody solution (for details see table above) diluted in blocking buffer overnight at 4^{o}C with gentle agitation.
6. Following two washes for 5 minutes each and one wash for 10 minutes at room temperature with TBST, the membrane was incubated in the secondary antibody (for details see table above) diluted in blocking buffer for 1 hour at room temperature with gentle agitation.
7. The membrane was then washed three times for 5 minutes and then 8 minutes with TBST at room temperature.
8. After draining away excess TBST, signals were detected with the detection kit detailed above, the blots exposed on X-ray film and the final images obtained using PS software.

EXPERIMENTAL NOTES

Under these experimental conditions, TAU P76 exhibits an immunoreactive band in the Human cell line, Human brain and Mouse brain lysates at around the expected MW of 79kDa. The lower MW bands seen in the Mouse brain lysate may represent TAU isoforms. TAU P80 also exhibits this band in the Human cell line lysate although this is not seen in either the Human or Mouse brain lysates. Various immunoreactive bands are observed using these two and the other antibodies which may represent TAU isoforms but the majority seem inconsistent with the expected MWs of TAU isoforms so may be non-specific background.

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 (star performer)
-1x Santa Cruz Biotechnology sc-5587 (high reviews)
-1x Abcam ab64193 (high reviews)
http://www.antibodyresource.com/superstars

Images of Superstar TAU antibodies: