ANTIBODY TESTING REPORT

SUMMARY

Antigen: TH (Uniprot# P07101)

Method tested: Immunohistochemistry

Laboratory ID: LAB07

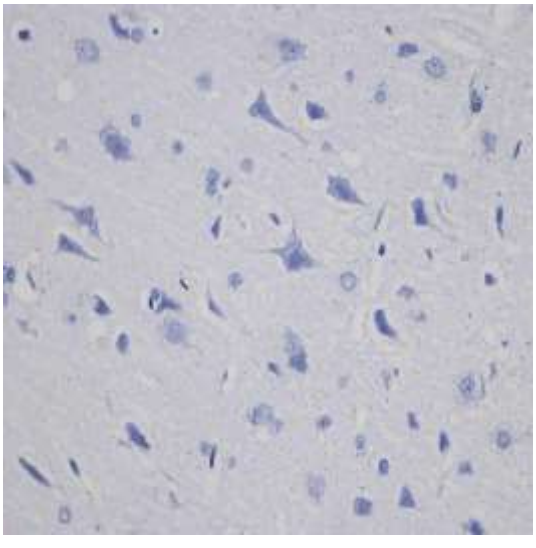
Project ID: AR147

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.

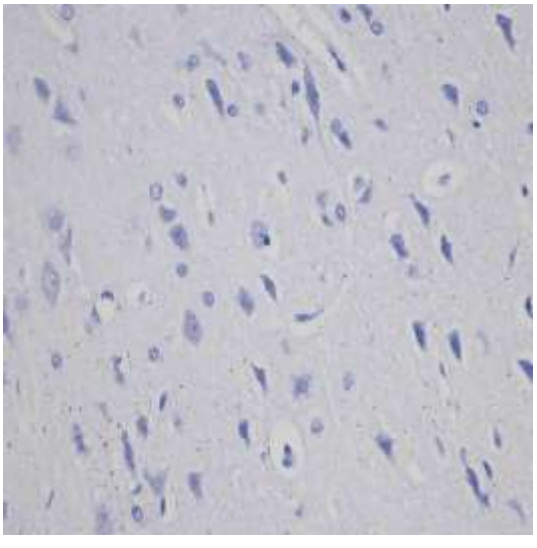
For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID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

RESULTS

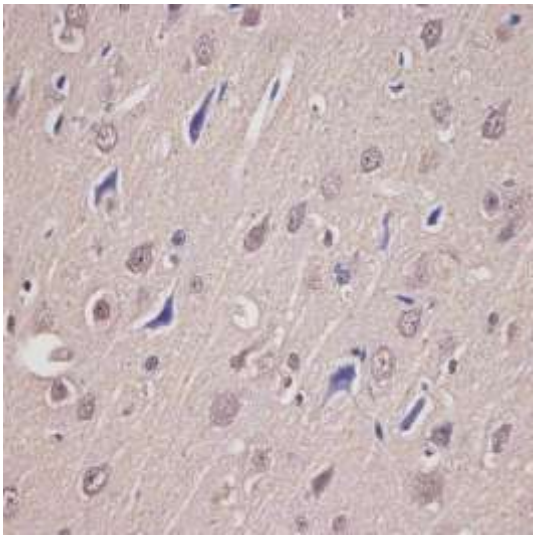
Immunohistochemical analysis of formalin fixed, paraffin embedded Rat brain tissue using various anti-TH antibodies and isotype controls (see Method section for more detail).



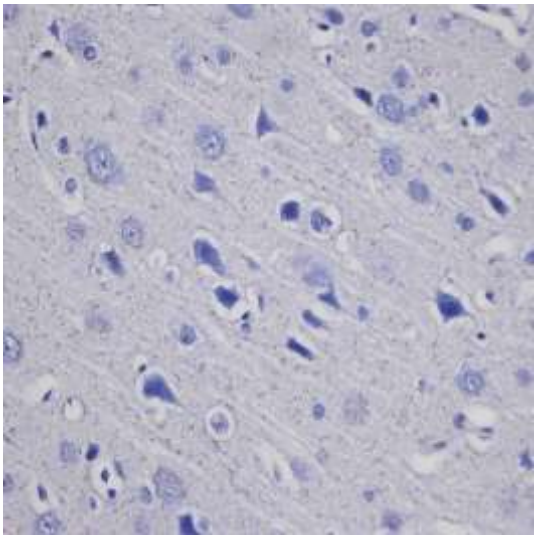
Antibody : TH M141 at 1/5000



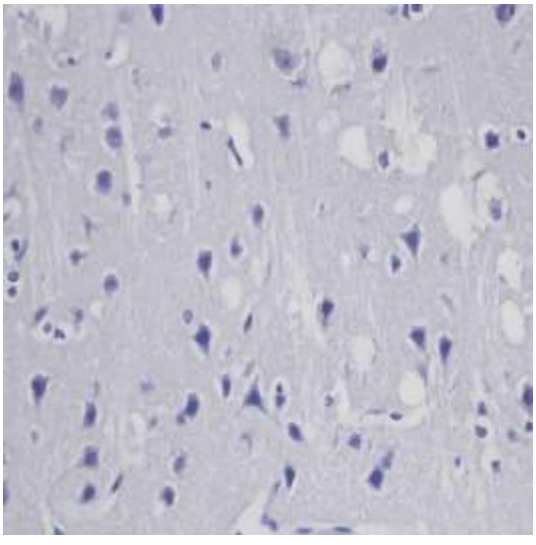
Antibody : Isotype control



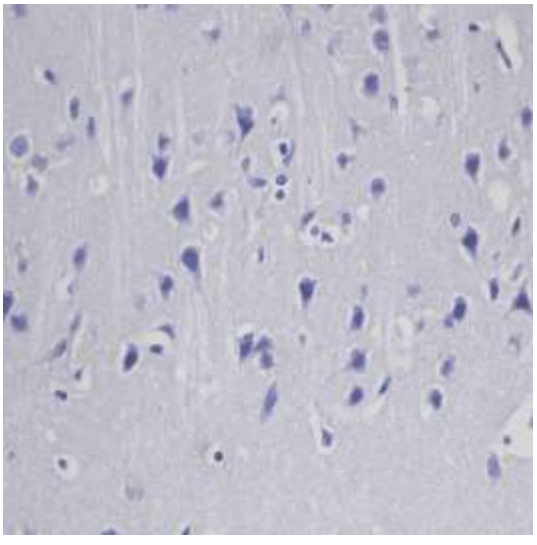
Antibody : TH M144 at 1/100



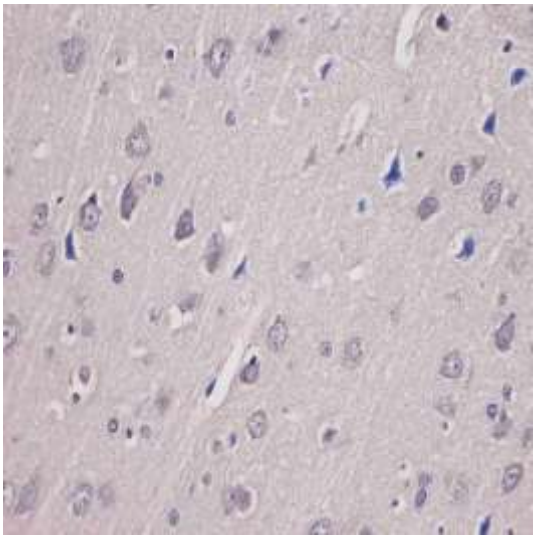
Antibody : Isotype control



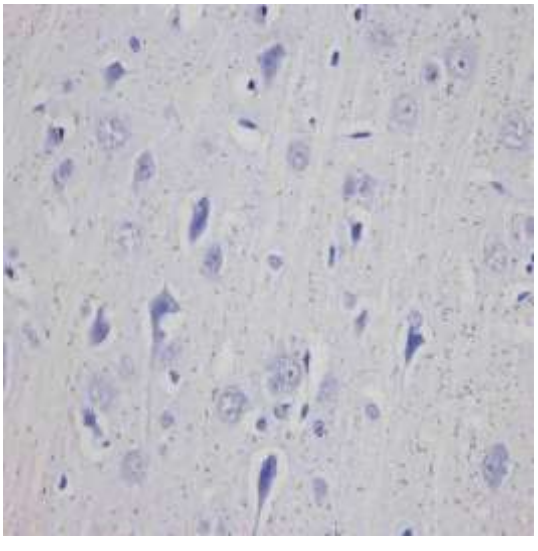
Antibody : TH P139 at 1/2000



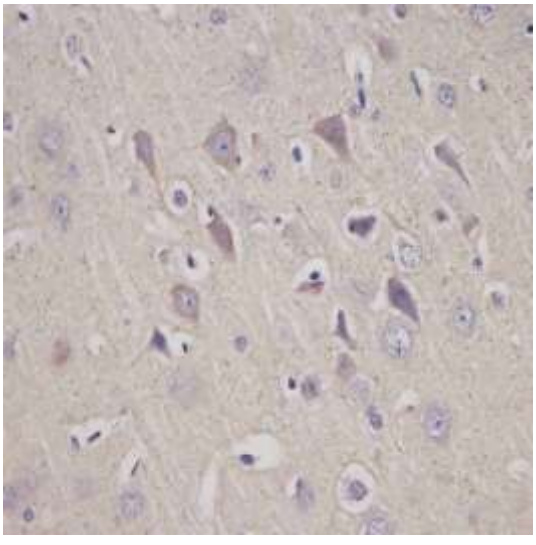
Antibody : Isotype control



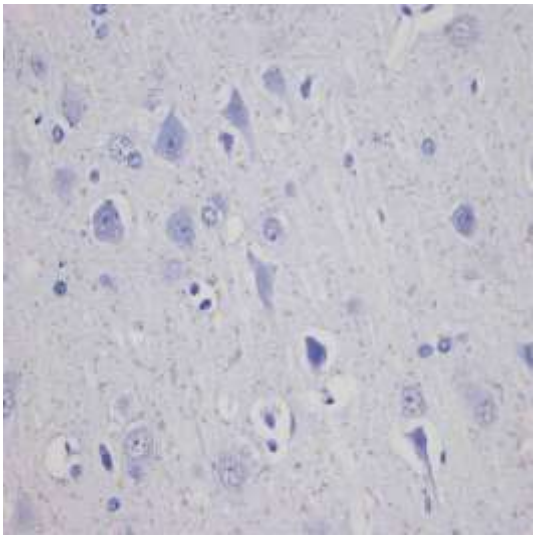
Antibody : TH P150 at 1/2000



Antibody : Isotype control



Antibody : TH P159 at 1/100



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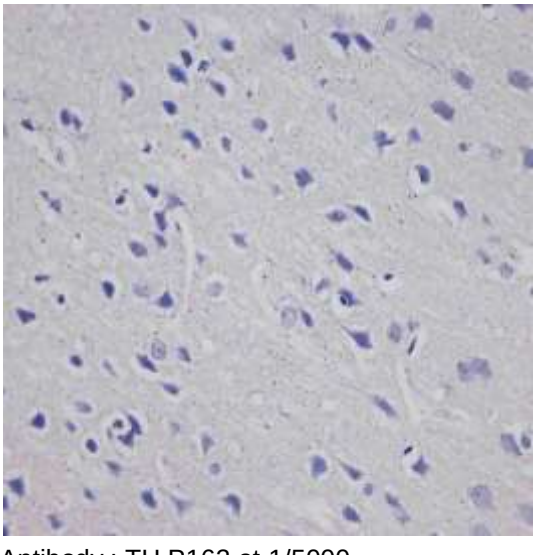
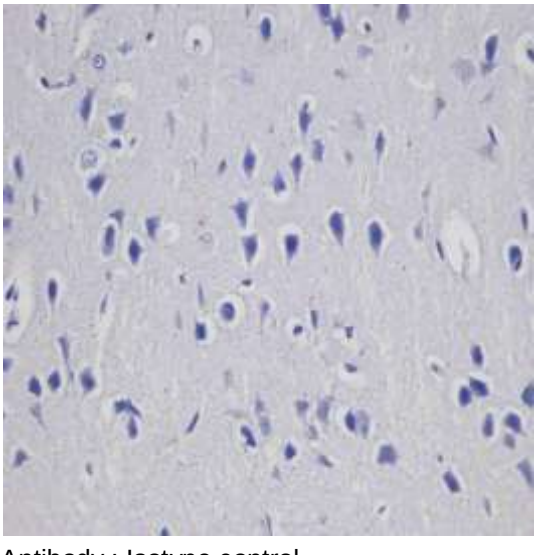
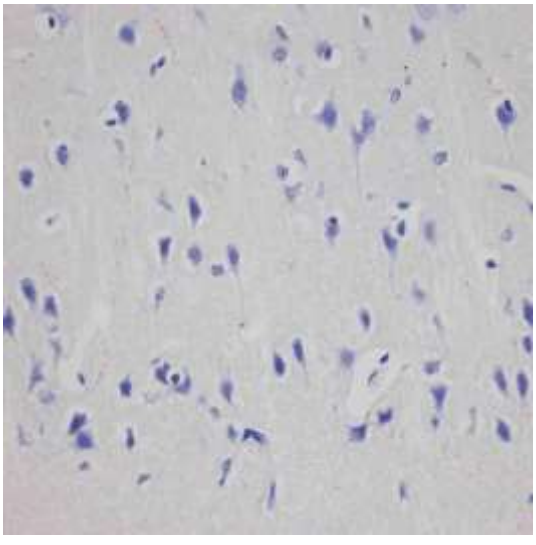


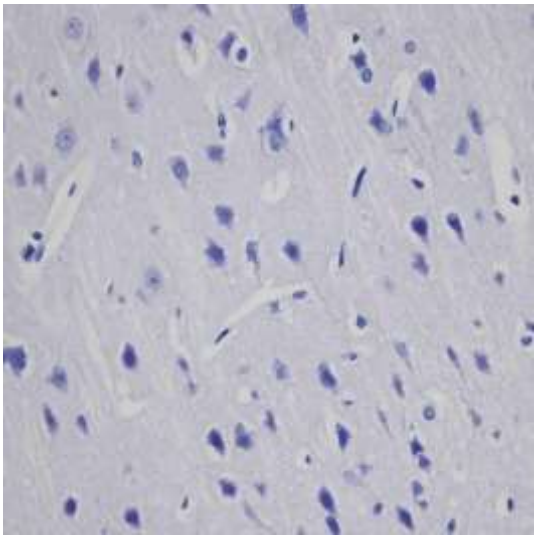
Antibody : TH P162 at 1/5000



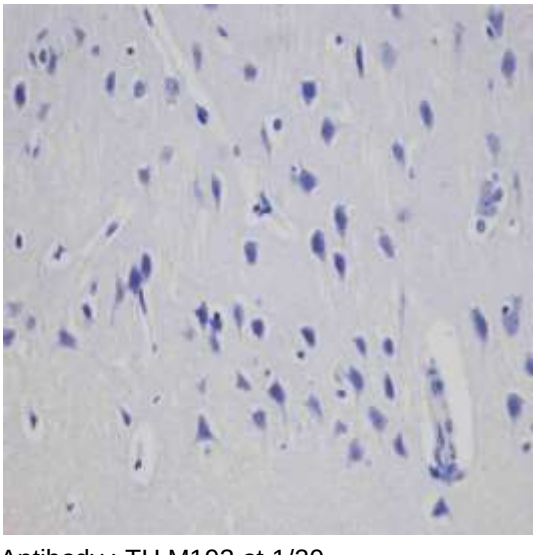
Antibody : Isotype control



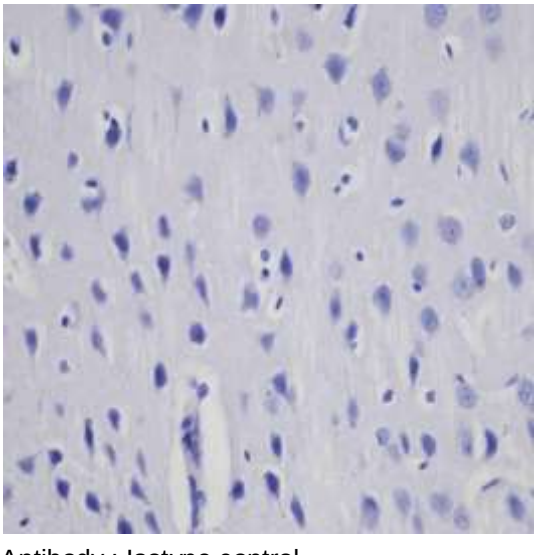
Antibody : TH P165 at 1/1000



Antibody : Isotype control



Antibody : TH M193 at 1/20



Antibody : Isotype control

METHOD

Antibodies

Primary antibody	Secondary antibody	Isotype Control
TH M141 at 1/5000 (Supplier 38)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG1 Isotype Control (ThermoFisher Scientific, SA1-12182) at 1/5000
TH M144 at 1/100 (Supplier 22)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/100
TH P139 at 1/2000 (Supplier 08)	Peroxidase AffiniPure Goat Anti-Chicken IgY (IgG) (H+L) (Jackson ImmunoResearch 103-035-155) at 1/300	ChromPure Chicken IgY (IgG), whole molecule (Jackson ImmunoResearch, 003-000-003) at 1/2000
TH P150 at 1/2000 (Supplier 31)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/2000
TH P159 at 1/100 (Novus)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
TH P162 at 1/5000 (Supplier 39)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/5000
TH P165 at 1/1000 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/1000
TH M193 at 1/20 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/20

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

PROTOCOL

Immunhistochemical analysis of formalin fixed, paraffin embedded Rat brain was performed using Nikon's DS-Ri1 system.

1. Tissue slides were preheated in convection oven at 60°C for 30min**.**
2. Deparaffinization was performed by immersing the slides three times in xylene for 10 minutes each time, followed by 5 minutes in 100% ethanol; then 5 minutes in 95% ethanol, 5 minutes in 80% ethanol, 5 minutes in 70% ethanol and finally three washes in distilled water of 5 minutes per wash.
3. An antigen retrieval procedure was then performed by heating the tissue slides, immersed in 10mM sodium citrate buffer, pH 6.0, in a microwave for 8 – 15 minutes. The slices were then allowed to cool at room temperature for 20 – 30 minutes.
4. Endogenous peroxidases were blocked by soaking the slides in 3% hydrogen peroxide-methanol for 15 minutes at room temperature, followed by two washes in distilled water of 5 minutes per wash and one wash of 5 minutes in PBS. Blocking was completed by incubating the slices in 3% BSA in PBS for 30 minutes at room temperature.
5. The slides were then immersed in the primary antibody solution diluted in PBS containing 3% BSA at 37^{o}C for 1 hour or overnight at 4^{o}C in a humidified chamber. Each antibody was diluted according to the working range suggested by the supplier (for details see table above).
6. Following three washes for 5 minutes each wash at room temperature with PBS-Tween (PBST), the slides were incubated in secondary antibody. This was either in the biotinylated secondary antibody from the HRP Anti-Polyvalent kit for 30 minutes at 37^{o}C in a humidified chamber or if the Peroxidase-conjugated AffiniPure Goat Anti-Chicken IgG was used, the slide was incubated for 60 minutes at 37^{o}C in a humidified chamber (for details see table above).
7. After removal of the secondary antibody solution, the slides were washed three times for 5 minutes per wash in PBST and then incubated in Streptavidin-HRP solution for 10 minutes at 37^{o}C if the HRP Polyvalent kit was used or on to step 8 if the Peroxidase-conjugated AffiniPure Goat Anti-Chicken IgG was used as the secondary antibody.
8. DAB staining solution was immediately added and the slides observed until the desired colour change was obtained (typically between 30 seconds and 5 minutes). After draining away excess solution, the sides were placed into distilled water for 5 minutes.
9. The slides were then incubated with haematoxylin for 3 minutes as counterstain.
10. Following three washes with distilled water, the slides were dehydrated by subsequent 5 minute washes in 70% ethanol, 80% ethanol, 95% ethanol, twice with 100% ethanol and two 10 minute washes with xylene. A coverslip was secured on each slide
11. The resulting staining of the tissue was observed and recorded.

EXPERIMENTAL NOTES

Under these experimental conditions, TH P159 exhibits cytoplasm staining in the Rat brain tissue which is consistent with the expected location. TH M144 and TH P150 have some staining but this does not appear to be exclusively in the cytoplasm whilst no staining is seen using TH M141, TH M193, TH P139, TH P162 or TH P165. These antibodies could be further investigated at higher concentrations.

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 $358, €340, £252.

The TH Superstarter Antibody Panel consists of:

1 x	AB152	(Millipore)
1 x	22941	(Immunostar)
1 x	T1299	(Sigma-Aldrich)
1 x	NBP2-42212	(Novus Biologicals)

http://www.antibodyresource.com/superstars

Images of Superstar TH antibodies: