Validation Report #029576

Validation Date: 01/11/14

Summary

Antigen	Amyloid beta A4 precursor protein (APP)
Catalog number	ABIN197433
Supplier	Acris
Supplier catalog number	ap02715pu-s
Lot number	8715
Method validated	Immunofluorescence
Laboratory	Molecular Pathology Core
Validation number	29576
Positive Control	Human brain
Negative Control	Human liver
Notes	Signal was detected in positive control tissue, and no signal was seen in negative control tissue.

Full Methods

Primary Antibody
- Antibody: Amyloid beta A4 precursor protein (APP)
- Catalog number: ABIN197433
- Supplier: Acris
- Supplier catalog number: ap02715pu-s
- Lot number: 8715

Isotype Control Antibody
- Antibody: Rabbit IgG control
- Supplier: Vector
- Catalog number: I-1000
- Lot number: T0503

Secondary Antibody
- Antibody: AlexaFluor 488 goat anti-Rabbit IgG
- Catalog number: A11034
- Supplier: Invitrogen
- Lot number: 702323

Controls
- Positive control: human brain (specimen known to contain the target protein) from Molecular Pathology Core.
- Negative Control: human liver (specimen known to not contain the target protein or express low level) from Molecular Pathology Core.
- Primary antibody isotype control: human brain treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: human brain treated with secondary antibody only (no primary antibody).

Protocol
- Sections were deparaffinized and rehydrated.
- Sections were heated to 98°C for 20 min in citrate buffer pH 6.0 (Biogenex, HK086-9K) for antigen retrieval and cooled down for 20 min on the bench.
- Sections were blocked in 10% NGS (normal goat serum) for 20 min at room temperature.
- Sections were washed x 2 in 1xTBS buffer.
- Sections were incubated with primary antibody diluted 1:100 in Antibody Diluent (Invitrogen, 003218) at 4°C overnight.
- Sections were washed x 2 in 1xTBS buffer.
- Sections were incubated with AlexaFluor 488 Goat anti-Rabbit IgG 1:1000 for 60 min.
- Sections were washed x 3 in 1xTBS buffer.
- Sections were mounted with DAPI (Invitrogen, Prolong Gold antifade reagent with DAPI) and coverslipped.
- Sections were photographed with a Zeiss Axioskop2 microscope

Experimental Notes

None

Figures

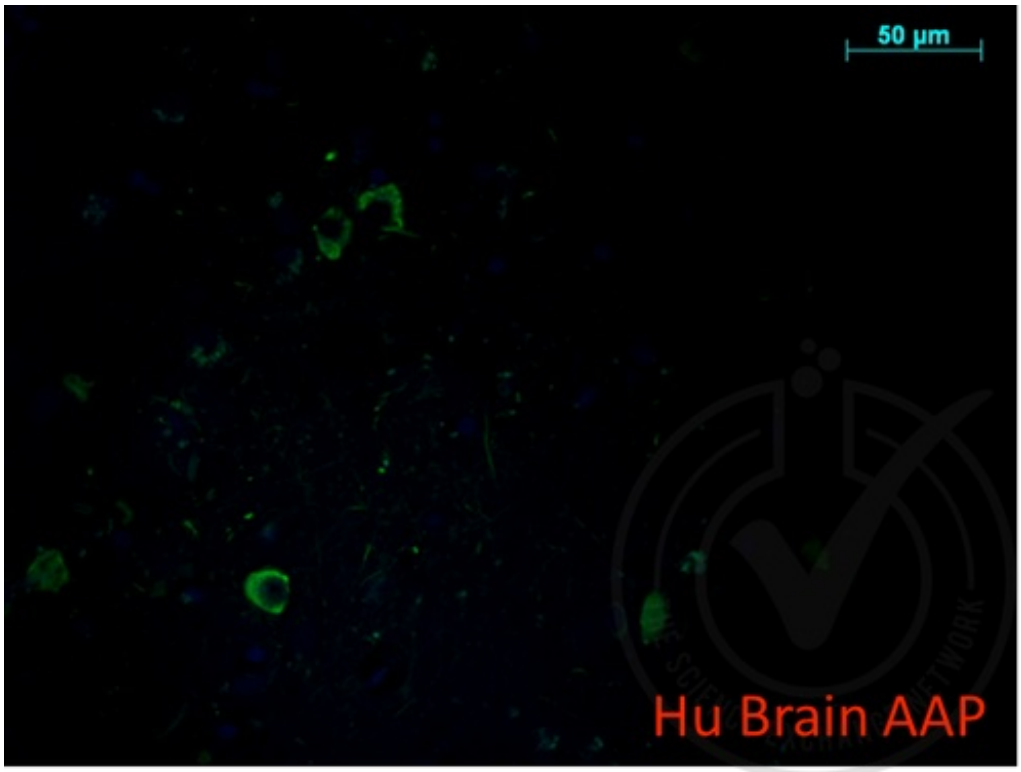

Figure 1. Micrograph image of positive control (human brain FFPE tissue). APP staining appears in green.

Figure 2: micrograph image of negative control (human liver FFPE tissue) stained with APP antibody.

Figure 3: micrograph image of isotype control (rabbit IgG isotype control antibody on human brain FFPE tissue).

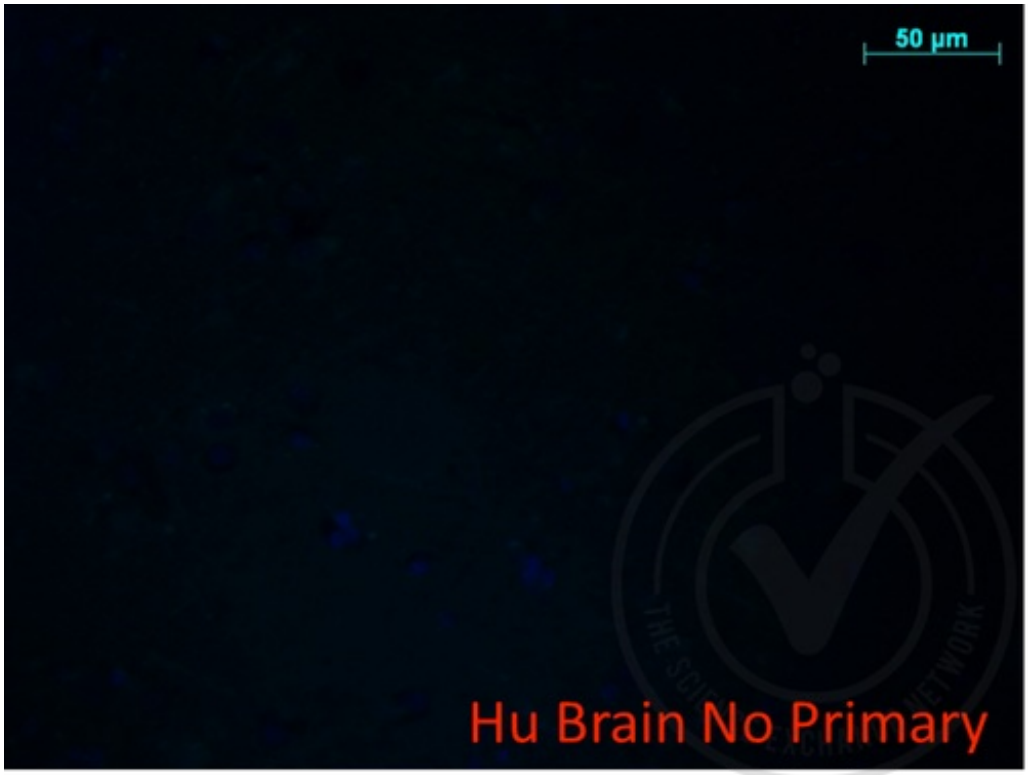

Figure 4: micrograph image of secondary antibody only control (no primary antibody on human brain FFPE tissue).