Validation Report #028758

Validation Date: 09/12/13

Summary

Antigen	Clathrin (AA 4-171, Heavy Chain)
Catalog number	ABIN968006
Supplier	BD Bioscience
Supplier catalog number	610499
Lot number	14494
Method validated	Immunohistochemistry
Laboratory	Reveal Biosciences
Validation number	28758
Positive Control	Brain
Negative Control	White adipose tissue
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody
- Antibody: Clathrin (AA 4-171,Heavy Chain)
- Catalog number: ABIN968006
- Supplier: BD Bioscience
- Supplier catalog number: 610499
- Lot number: 14494

Isotype Control Antibody
- Antibody: Mouse IgG1 kappa isotype control
- Supplier: Antibodies Online
- Catalog number: ABIN1379864
- Lot number: 2188837

Secondary Antibody
- Antibody: Rabbit anti-mouse IgG HRP
- Supplier: Antibodies Online
- Catalog number: ABIN1384775
- Lot number: YYDW56G

Controls
- Positive control: rat cerebellum (specimen known to contain the target protein) from Explora BioLabs.
- Negative Control: white adipose tissue (specimen known to not contain the target protein) from Explora BioLabs.
- Primary antibody isotype control: rat cerebellum treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: rat cerebellum treated with secondary antibody only (no primary antibody).

Protocol

Immunohistochemistry was performed on a Leica Bond automated immunostainer.
- Sections were deparaffinized with Novocastra Bond Dewax Solution and rehydrated into Leica Bond Wash Buffer.
- Sections were heated to 98°C for 20 minutes in Tris buffer pH 9.0 (ER2; Leica) for antigen retrieval.
- Sections were blocked in 3% normal goat serum plus 0.1 % Triton-X100 for 10 min at room temperature.
- Sections were washed x 3 in Leica Bond Wash Buffer.
- Sections were incubated with primary antibody diluted 1:100 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25885-05) for 60 min at room temperature.
- Sections were washed x 3 in Leica Bond Wash Buffer.
- Sections were incubated with secondary antibody diluted 1:100 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25885-05) for 60 min at room temperature..
- Sections were washed x 4 in Leica Bond Wash Buffer.
- Sections were washed x 1 in Distilled Water. Sections were incubated with Peroxide Block (Leica) for 10 min to block endogenous peroxidase.
- Sections were washed x 4 in Leica Bond Wash Buffer.
- Sections were incubated with DAB chromogenic substrate (Leica) for 10 min at RT.
- Sections were washed x 3 in Distilled Water.
- Sections were counterstained with hematoxylin (Leica) for 2 min.
- Sections were washed x 1 in Distilled Water.
- Sections were washed x 1 in Leica Bond Wash Buffer.
- Sections were washed x 1 in Distilled Water.
- Sections were dehydrated, mounted and photographed under a light microscope.

Experimental Notes
- Nothing to note.

Figures

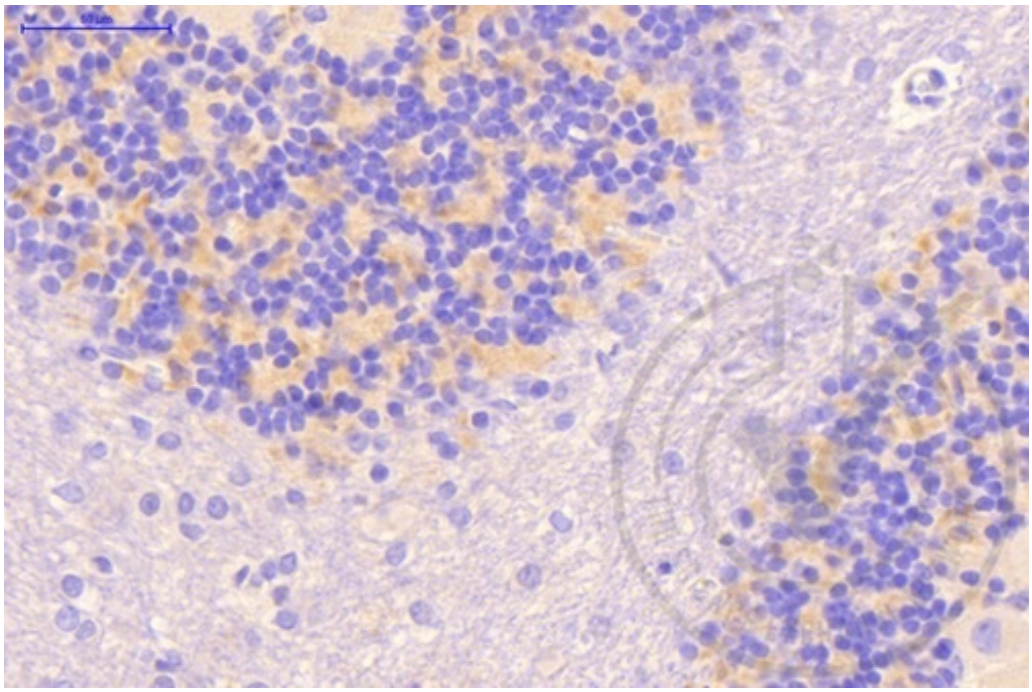

Figure 1: Cerebellum stained with anti-Clathrin (brown) and counterstained in blue.

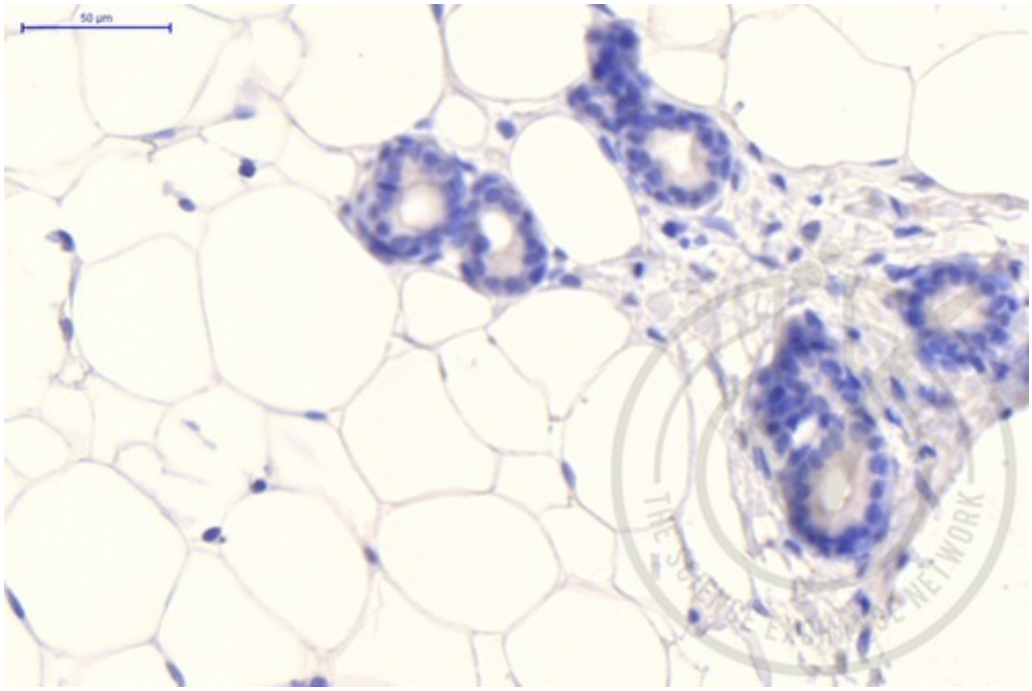

Figure 2: White adipose tissue stained with anti-Clathrin (brown) and counterstained in blue.

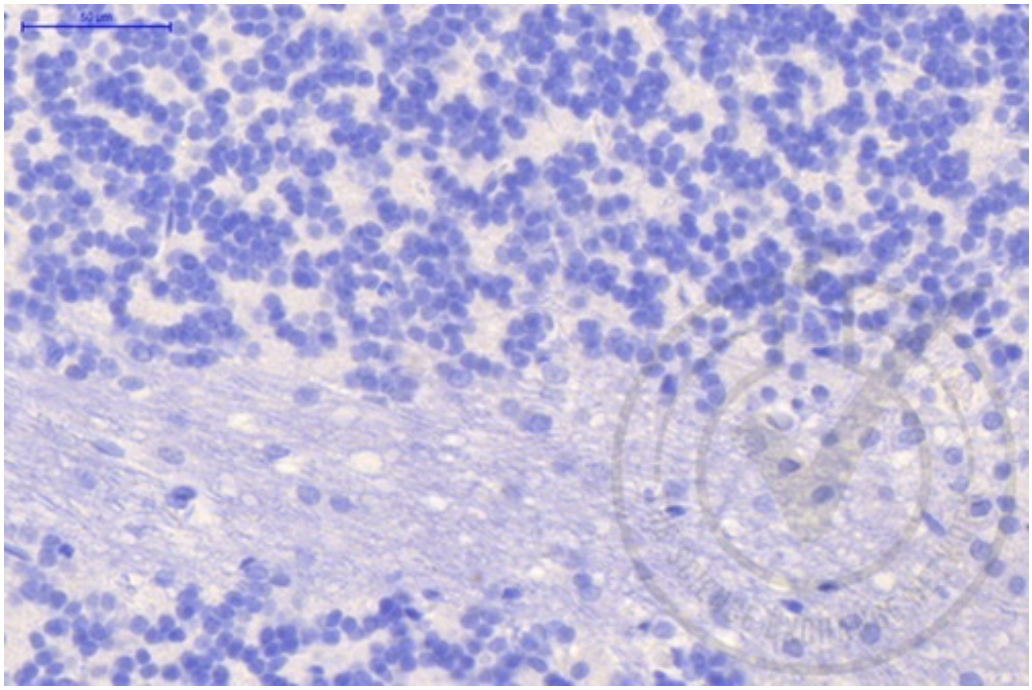

Figure 3: Cerebellum stained with isotype control (brown) and counterstained in blue.

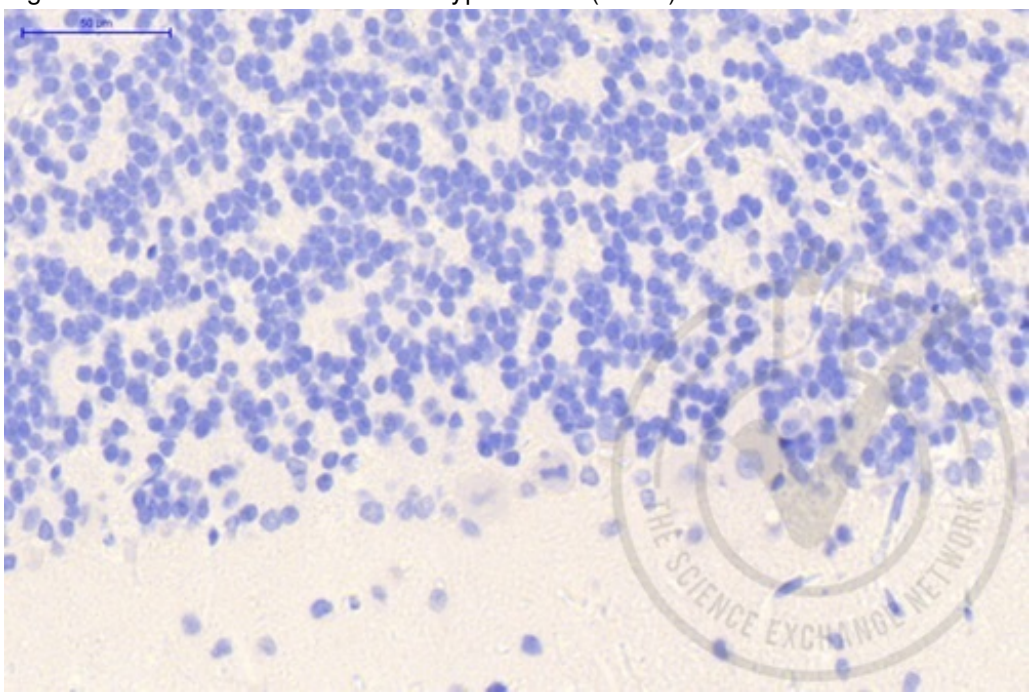

Figure 1: Cerebellum stained with secondary antibody only (brown) and counterstained in blue.