Datasheet for ABIN968006

anti-Clathrin antibody (AA 4-171)

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Overview

Quantity:	50 µg
Target:	Clathrin
Binding Specificity:	AA 4-171
Reactivity:	Human, Mouse, Rat, Chicken, Dog
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Clathrin antibody is un-conjugated
Application:	Immunohistochemistry (IHC), Western Blotting (WB), Immunofluorescence (IF), Immunoprecipitation (IP), BioImaging (BI)

Product Details

Immunogen:	Rat Clathrin Heavy Chain aa. 4-171
Clone:	23-Clathrin Heavy Chain
Isotype:	IgG1
Cross-Reactivity:	Human, Chicken, Dog (Canine), Mouse (Murine)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing. 4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

Product Details

Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	Clathrin
Abstract:	Clathrin Products
Background:	Clathrin is the major protein component in the coat formed around pits and vesicles involved in receptor-mediated endocytosis. Clathrin forms a non-covalently bound triskelion structure composed of three heavy chains (192 kDa each) and three light chains (23-25 kDa each). Each leg of the triskelion structure contains one heavy and one light chain. The three heavy chains forming the triskelion structure are attached at their respective proximal ends like spokes on a wheel. Clathrin heavy chain is composed of a terminal globular domain, a distal segment containing several areas sensitive to enzymatic cleavage, and a proximal segment which contains a light chain binding site. The proximal and distal domains are connected by a joint segment at which there is a sharp bend in the heavy chains of fully-assembled triskelia. Although the calculated molecular weight is 192 kDa, clathrin heavy chain migrates at approximately 180 kDa.
Molecular Weight:	180 kDa

Application Details

Application Notes:	Immunofluorescent Staining and Bioimaging Methanol Procedure for a 96 well plate: Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Triton-X 100 Procedure for a 96 well plate: Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS.

Application Details

Comment:	Related Products: ABIN968535
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.25 mg/ml
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20° C.

Publications

Product cited in:

Padilla, Chang, Pacheco-Rodriguez, Adamik, Moss, Vaughan: "Interaction of FK506-binding protein 13 with brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1): effects of FK506." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 100, Issue 5, pp. 2322-7, (2003) (PubMed).

Kalthoff, Groos, Kohl, Mahrhold, Ungewickell: "Clint: a novel clathrin-binding ENTH-domain protein at the Golgi." in: **Molecular biology of the cell**, Vol. 13, Issue 11, pp. 4060-73, (2002) (PubMed).

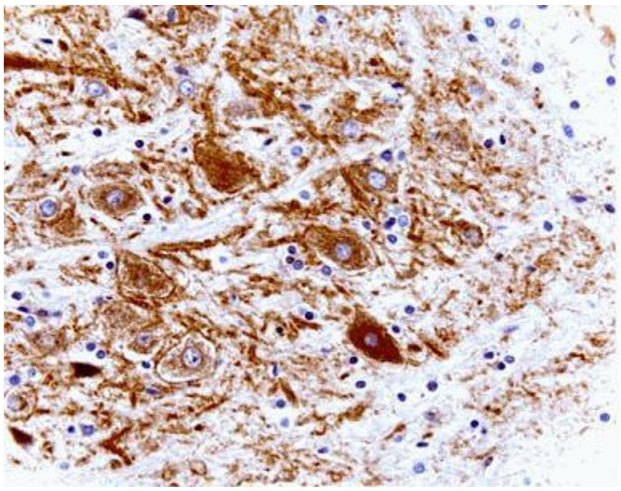
van Kerkhof, Sachse, Klumperman, Strous: "Growth hormone receptor ubiquitination coincides with recruitment to clathrin-coated membrane domains." in: **The Journal of biological chemistry**, Vol. 276, Issue 6, pp. 3778-84, (2001) (PubMed).

Okamoto, Karam, Jeng, Forte, Goldenring: "Identification of clathrin and clathrin adaptors on tubulovesicles of gastric acid secretory (oxyntic) cells." in: **The American journal of physiology**, Vol. 274, Issue 4 Pt 1, pp. C1017-29, (1998) (PubMed).

Liu, Wong, Craik, Brodsky: "Regulation of clathrin assembly and trimerization defined using

recombinant triskelion hubs." in: **Cell**, Vol. 83, Issue 2, pp. 257-67, (1995) (PubMed).

Images

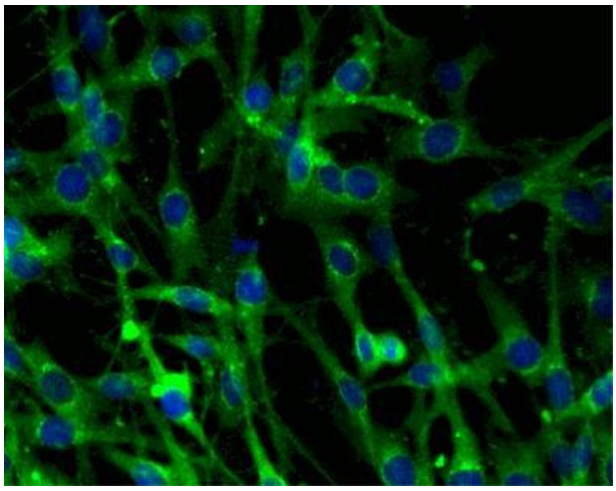


Immunohistochemistry (Paraffin-embedded Sections)

Image 1. Rat cerebellum, zinc-fixed paraffin embedded tissue (left), 40X

Western Blotting

Image 2. Western blot analysis of Clathrin Heavy Chain on HeLa cell lysate (center). Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of anti-Clathrin Heavy Chain.



Immunofluorescence

Image 3. Immunofluorescent staining of C6 cells (right). Cells were seeded in a collagen coated 384 well imaging plate at ~ 6,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol and the anti-Clathrin Heavy chain antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). The image was taken on a Pathway 855 or 435 imager using a 20x objective. This antibody also stained SH-SY5Y, SK-N-SH cells using both the Triton X100 and methanol fix/perm protocols.

Successfully validated (Immunohistochemistry (IHC))

by Reveal Biosciences

Report Number: 028758

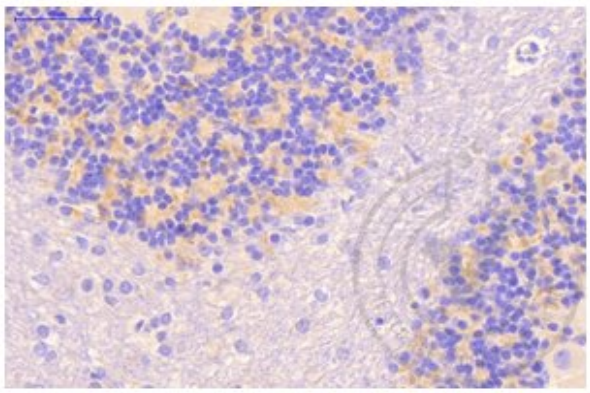
Date: Sep 12 2013

Lot Number:	14494
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Brain
Negative Control:	White adipose tissue
Notes:	Signal was detected in positive control sample and not in negative control sample.
Primary Antibody:	- Antibody: Clathrin (AA 4-171,Heavy Chain) - Catalog number: ABIN968006 - Supplier: BD Bioscience - Supplier catalog number: 610499 - Lot number: 14494
Secondary Antibody:	- Antibody: Rabbit anti-mouse IgG HRP - Supplier: Antibodies Online - Catalog number: ABIN1384775 - Lot number: YYDW56G
Isotype:	- Antibody: Mouse IgG1 kappa isotype control - Supplier: Antibodies Online - Catalog number: ABIN1379864 - Lot number: 2188837
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.

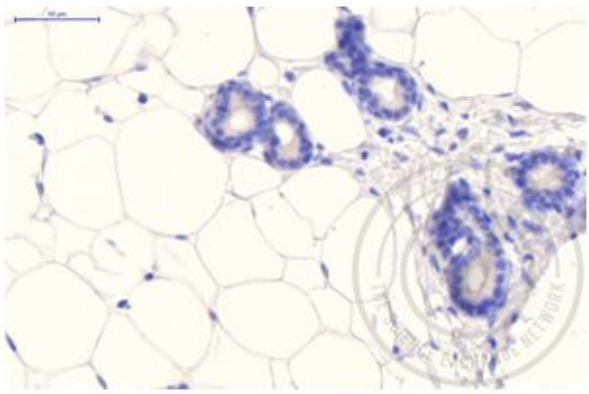
Experimental Notes:	- Nothing to note.

Images for Validation report #028758



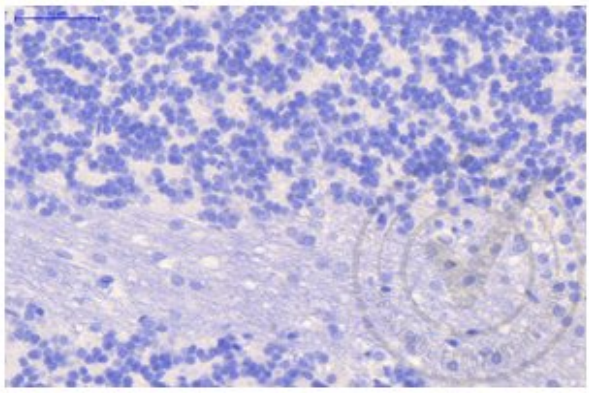
Validation image no. 1 for anti-Clathrin (AA 4-171) antibody (ABIN968006)

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



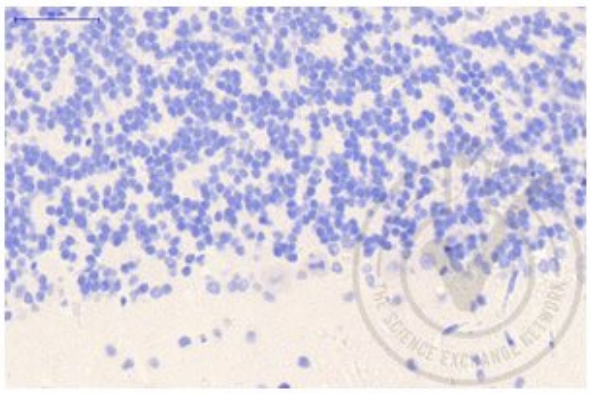
Validation image no. 2 for anti-Clathrin (AA 4-171) antibody (ABIN968006)

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



Validation image no. 3 for anti-Clathrin (AA 4-171) antibody (ABIN968006)

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



Validation image no. 4 for anti-Clathrin (AA 4-171) antibody (ABIN968006)

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