Technical Data Sheet

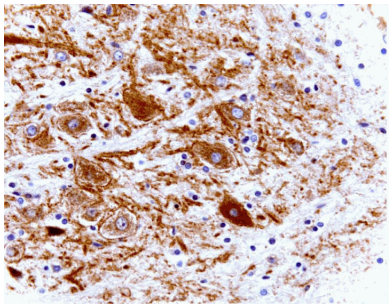
Purified Mouse Anti-Clathrin Heavy Chain

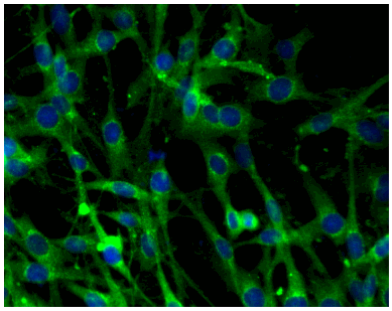
Product Information

Material Number:	**610500**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	23/Clathrin Heavy Chain
Immunogen:	Rat Clathrin Heavy Chain aa. 4-171
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	180 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

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.





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

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.*

Immunofluorescent staining of C6 cells (right). *Cells were seeded in a collagen coated 384 well imaging plate (Cat. # 353962) at ~ 6,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) 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 (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

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Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

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. Image sample.

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. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
353962	BD Falcon™ 384-well Imaging Plate	NA	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols 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.

References

Kalthoff C, Groos S, Kohl R, Mahrhold S, Ungewickell EJ. Clint: a novel clathrin-binding ENTH-domain protein at the Golgi. *Mol Biol Cell.* 2002; 13(11):4060-4073. (Clone-specific: Immunofluorescence, Western blot)

Liu SH, Wong ML, Craik CS, Brodsky FM. Regulation of clathrin assembly and trimerization defined using recombinant triskelion hubs. *Cell.* 1995; 83(2):257-267. (Biology)

Okamoto CT, Karam SM, Jeng YY, Forte JG, Goldenring JR. Identification of clathrin and clathrin adaptors on tubulovesicles of gastric acid secretory (oxyntic) cells. *Am J Physiol.* 1998; 274(1):C1017-C1029. (Clone-specific: Immunohistochemistry, Western blot)

Padilla PI, Chang MJ, Pacheco-Rodriguez G, Adamik R, Moss J, Vaughan M. Interaction of FK506-binding protein 13 with brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1): effects of FK506. *Proc Natl Acad Sci U S A.* 2003; 100(5):2322-2327. (Clone-specific: Immunoprecipitation, Western blot)

van Kerkhof P, Sachse M, Klumperman J, Strous GJ. Growth hormone receptor ubiquitination coincides with recruitment to clathrin-coated membrane domains. *J Biol Chem.* 2001; 276(6):3778-3784. (Clone-specific: Electron microscopy)