

Product Information

Monoclonal Anti-Clathrin Heavy Chain antibody produced in mouse

clone TD.1, purified from hybridoma cell culture

Catalog Number **C1860**

Product Description

Monoclonal Anti-Clathrin Heavy Chain (mouse IgG1 isotype) is derived from the TD.1 hybridoma produced by the fusion of mouse myeloma cells and lymph node cells from a BALB/c mouse immunized with a globular N-terminal domain of bovine clathrin heavy chain.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-Clathrin Heavy Chain reacts specifically with the clathrin heavy chain (CHC17), and does not cross-react with the muscle isoform CHC22.² The epitope recognized by the antibody resides within amino acids 207-264 of bovine CHC.¹ The product is useful in immunoblotting¹⁻⁵ (192 kDa, apparent size in SDS-PAGE is approximately 180 kDa). It is reported to be non-reactive with native clathrin in immunocytochemistry, and immunoprecipitation (but some staining is seen in Rat1 cells, following 4% paraformaldehyde-0.5% Triton™ X-100 fixation). Cross-reactivity has been observed with human,^{2,3} bovine,^{1,5} rat,⁴ and mouse³ CHC.

Endocytosis plays a critical role in cellular functions ranging from nutrient acquisition to synaptic transmission. Receptor-mediated endocytosis allows the specific removal of cell surface receptors and their cargo from the plasma membrane and targets them to endosomes, where they are sorted for downregulation or recycling.⁶ In multicellular organisms, different cell types utilize specialized pathways of intracellular membrane traffic to facilitate a specific physiological function. Such pathways are presumably mediated or enhanced by various tissue-specific membrane vesicle coat proteins.² It is widely assumed that coated vesicles mediate the selective transfer of molecules and membrane components between specific membranous organelles within cells. Clathrin is the main constituent of the polygonal network that forms the coat of coated vesicles and coated pits.

Different receptors have different requirements for their entry into coated pits. Those binding nutrients or inert cargo, such as the transferrin receptor, are internalized in the presence or absence of bound ligand, whereas signaling receptors, such as receptor tyrosine kinases

and G protein-coupled receptors, require ligand binding for uptake.³ In all cell types, clathrin-coated vesicles are responsible for receptor-mediated endocytosis at the plasma membrane and for the receptor-mediated sorting of lysosomal enzymes from *trans*-Golgi membranes to a pre-lysosomal compartment. In cells with a regulated secretory pathway, clathrin-coated membranes are involved in the formation of secretory granules and in the rapid uptake of plasma membrane following degranulation. Thus, in addition to their constitutive functions of bringing nutrients into the cell and maintaining the degradative pathway of the cell, clathrin-coated vesicles play an important role in hormone secretion and mechanism of action.

A number of protein-protein and protein-lipid interactions underlie the assembly of the clathrin-based endocytic machine. In addition to clathrin, AP-2, and in neurons AP180, the proteins include dynamin and amphiphysin. Accessory cytosolic proteins include synaptojanin I, an inositol 5-phosphatase, and Eps15.⁴

Formation of clathrin-coated pits initiates the budding of clathrin-coated vesicles from membranes.⁷ These vesicles selectively remove receptors from a donor membrane and facilitate their transport to an intracellular target membrane with which the uncoated vesicle fuses. At each site, clathrin associates indirectly with the membrane by binding to an adaptor protein (AP) which interacts with the cytoplasmic domains of transmembrane receptors. Polymerization of clathrin then concentrates adaptors, associated receptors and their bound ligands into a coated vesicles.⁶

Clathrin-coated pits form at the plasma membrane and the *trans*-Golgi network (TGN).^{5,6,8} Coated pits at each intracellular location have different adaptors. The AP1 adaptor is found in the TGN and the AP2 adaptor is found at the plasma membrane, where clathrin-coated vesicles play a role in biogenesis of intracellular organelles or in receptor-mediated endocytosis, respectively.⁵ Because adaptors are restricted in intracellular location, the adaptor-membrane interaction is believed to determine where clathrin-coated pits are formed.⁵

Clathrin has a three-legged structure termed triskelion. The triskelion consists of three heavy chain polypeptides, each bound to a light chain (LC) of which there are two different types, LCa and LCb.¹ The clathrin heavy chain is known to provide the backbone for the clathrin lattice. CHC is composed of a terminal globular domain, a distal segment and a proximal segment containing a LC binding site. A clathrin homologue encoded on human chromosome 22 (CHC22) displays distinct biochemistry, distribution, and function compared with conventional clathrin heavy chain (CHC17), encoded on chromosome 17.² Clathrin light chains (25-29 kDa, appearing as single or double bands of 25-36 kDa in SDS-PAGE)⁹ contain regulatory domains that influence the recruitment, assembly, and disassembly of clathrin within the cell.

Various cell types express both forms of clathrin LC in different relative levels. LCs are randomly distributed on clathrin triskelion, resulting in four types of clathrin triskelion with different LC composition (3LCa, 2LCa-1LCb, 2LCb-1LCa, 3LCb).⁹ In most cells the LCa form is dominant. In contrast, cells with a regulated secretory pathway contain more LCb than LCa, suggesting that LCb may play a role in the specialized clathrin functions in these cells. Neuronal cells, express splicing variants of both LCa and LCb.⁹ A distinguishing feature of the LCb light chain is its ability to be phosphorylated by a casein kinase, at a site which is not present in the LCa sequence.

Monoclonal antibody reacting specifically with clathrin heavy chain is a useful tool to study the mechanisms that underlie clathrin-mediated membrane traffic during secretion, hormonal stimulation, and protein phosphorylation.

Reagent

Supplied at ~2 mg/ml in a solution of 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 2-4 µg/ml is determined using a whole extract of cultured rat adrenal pheochromocytoma PC-12 cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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