MONOCLONAL ANTI-ASSEMBLY PROTEIN AP180 (AP-3)
CLONE AP180-I
Mouse Ascites Fluid

Product No. **A4825**
Lot 014H4832

Monoclonal Anti-Assembly Protein AP180 (AP-3) (mouse IgG2b isotype) is derived from the AP180-I hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A membrane protein fraction from purified bovine brain coated vesicles was used as the immunogen.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is provided as ascites fluid with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity
Monoclonal Anti-Assembly Protein AP180 (AP-3) reacts with the AP180 polypeptide (180 kD) and with the proteolytic derivatives of AP180 (approx. 170 kD) in immunoblots of bovine and rat brain preparations.[1-4] The antibody is reactive with human, bovine and rodent neuronal tissues and cultured cell lines. It may also be used for immunoprecipitation, immunoaffinity purification[1,3,4,5] and immunocytochemistry. For immunocytochemistry, fixation with acetone or methanol is recommended.

Working Dilution 1:10,000
The working dilution was determined by indirect immunoblotting using rat brain extract.

In order to obtain best results, it is recommended that each individual user determine working dilution by titration assay.

Description
Clathrin-coated membranes of eukaryotic cells participate in various intracellular transport processes, such as receptor-mediated endocytosis, the retrieval of membrane after exocytosis, the routing of lysosomal enzymes from the trans-Golgi network to a pre-lysosomal compartment, and the maturation of prohormone-filled exocytotic storage vesicles.[1,6] Vesicle transport is preceded by polymerization of clathrin protomers on the membrane leading to extended regular coats. These generate coated vesicles by a process of budding and severing. The ability to polymerize into regular coat structures is an inherent property of clathrin triskelia. However, it is strictly dependent on protein cofactors which are released from the coated vesicle membrane with clathrin in 0.5 M Tris. Four clathrin-binding proteins have been identified that support clathrin assembly *in vitro*. These are the AP-1 and AP-2 adaptor protein complexes, also known as HA-I (HA1) adaptor and HA-II (HA2) adaptor or Assembly Protein 1 (AP1) and Assembly Protein 2 (AP2), respectively, AP180 (AP-3) and auxilin.[2] Based on results obtained by molecular cloning and sequencing, the molecular weight of AP180 is 92 kD. Its sequence is unrelated to that of any other known protein.[7] Its expression is probably restricted to cells of neuronal origin and the two neuronal proteins AP-3 and NP185 were shown to be identical with AP180. AP180 migrates in standard Laemmli-type SDS PAGE as a 170-190 kD poly-peptide and is therefore difficult to resolve from the clathrin heavy chain. In polyacrylamide gels with a low proportion of crosslinker, AP180 separates well from the clathrin heavy chain, which behaves in this electrophoretic system like an approx. 150 kD polypeptide. AP180 binds to clathrin triskelia with a stoichiometry of one per clathrin triskelion (trimer of heavy chains) and thereby induces assembly of clathrin into polyhedral cages. Monoclonal antibodies which react specifically with coated vesicle proteins, are useful for studies on the intracellular distribution and structural relationship of clathrin-binding proteins.

Uses
Monoclonal Anti-Assembly Protein AP180 (AP-3) may be used for the localization of assembly protein AP180 using immunoblotting, immunocytochemistry, immunoprecipitation and for immunoaffinity purification.

Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

*Due to sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

References

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