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ProductInformation

MONOCLONAL ANTI-γ-ADAPTIN (AP-1)
Clone 100/3
Mouse Ascites Fluid

Product Number **A 4200**

Product Description

Monoclonal Anti-γ-Adaptin (AP-1) (mouse IgG2b isotype) is derived from the 100/3 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. AP-1 adaptor from bovine brain was used as the immunogen.[1] The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-γ-Adaptin (AP-1) reacts in immunoblotting with the 104 kDa polypeptide of the Golgi adaptor complex AP-1.[1-6] The antibody reacts with polypeptides of approximately 100 kDa in bovine liver, human heart fibroblasts, and Madin-Darby bovine kidney cultured cells (MDBK), but not with any components in the 110-115 kDa range from these sources, or from rat pheochromocytoma cultured cells (PC12), neuroblastoma or astrocytes. This suggests that the 110 and 115 kDa polypeptides may be specific variants that occur only in some cell types of brain. The antibody does not recognize the γ-subunit in rat and mouse. Using immunofluorescent staining on MDBK cells,[1] and African green monkey kidney cells,[6] the binding of the antibody appears to be largely confined to the trans-Golgi network. The product has been used for studies on the effects of Brefeldin A, which causes a rapid redistribution of coat proteins associated with the clathrin-coated vesicles that bud from the trans-Golgi network.[6] It may also be used for the immunoaffinity purification of the Golgi adaptor complex AP-1,[5] for immunoprecipitation applications,[1,3] and for immunoelectron microscopy.[7]

In eukaryotic cells, clathrin-coated vesicles mediate the selective internalization of cell-surface receptors for lysosomal enzymes from the trans-Golgi network to a prelysosomal compartment.

Besides clathrin, coated vesicle populations contain the adaptor complexes AP-1 and AP-2, also known as HA-I (HA1) adaptor and HA-II (HA2) adaptor or Assemble Protein 1 (AP1) and Assemble Protein 2 (AP2), respectively.[8] Interest has focused on adaptors because they are known to mediate the interaction between clathrin and components of the membrane. Their location between the clathrin shell and the membrane, as revealed by cryoelectron microscopy, is consistent with such a function. Under physiological conditions, *in vitro* adaptory proteins readily bind to clathrin and thereby induce its polymerization into regular polyhedral coat structures, very similar to the coats of coated vesicles. In the cell, clathrin-adaptor interactions proceed in a very controlled and specific manner, leading to coated structures each containing only one type of adaptor. Clathrin-coated membranes at or originating from the plasma membrane contain the AP-2 adaptor, while the AP-1 adaptor appears to be largely restricted to clathrin-coated membranes of the trans-Golgi network. Both the Golgi-associated AP-1 adaptor and the plasma-membrane associated AP-2 adaptor are heterotetrameric protein complexes. AP-1 adaptor consists of four subunits termed β_1 (formerly β', 110 kDa), γ (100 kDa), μ_1 (47 kDa), and σ_1 (20 kDa). β_1 and γ subunits (β_1 and γ adaptins) from neuronal sources behave in standard SDS-PAGE like 115 kDa and 104 kDa polypeptides, respectively. Similarly, the AP-2 is made up of two 100-110 kDa polypeptides, termed α and β_2 (formerly β) subunits, γ_2 (50 kDa), and σ_2 (17 kDa) subunit. α and β_2 subunits (α and β_2 adaptins) behave in standard SDS-PAGE like 100-112 kDa polypeptides. Four α-isoforms (αa1, αa2, αc1 and αc2) which are highly homologous in sequence have been described. β_1 and β_2 adaptins are highly homologous which suggests that the β-type adaptor chains may mediate the interaction of their adaptors with the highly conserved clathrin molecule. The α and β adaptins share only 25% overall identity.

The other adaptor subunits of the two adaptor complexes which appear to be unrelated, are believed to possess binding sites for different cargo molecules (receptors) and for proteins which might regulate the complex functions of the adaptors. Monoclonal antibodies reacting specifically against adaptor proteins are useful tools for studies on the intracellular distribution and structural relationship of adaptor complexes.Monoclonal Anti-γ-Adaptin (AP-1) may be used for localization of γ-adaptin in immunoblotting.

Reagents
The product is provided as ascites fluid with 0.1% sodium azide as a preservative.

Precautions and Disclaimer
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.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. 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.

Product Profile
A minimum working dilution of 1:100 was determined by indirect immunoblotting using bovine brain extract.

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

References

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JWM 07/02