

Product Information

MONOCLONAL ANTI- α -ADAPTIN (AP-2) CLONE 100/2 Mouse Ascites Fluid

Product No. A 4325

Product Description

Monoclonal Anti- α -Adaptin (AP-2) (mouse IgG2a isotype) is derived from the 100/2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. AP-2 adaptor polypeptides from bovine brain was used as the immunogen.¹ The isotype is determined using Sigma ImmunoType™ Kit (Product No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product No. ISO-2).

Monoclonal Anti- α -Adaptin (AP-2) reacts in immunoblotting of bovine and rat brain preparations, with the 105 and 110 α -subunits of adaptor complex AP-2.¹⁻⁵ 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 stains the intact α -adaptor, as well as the 37 and 40 kDa fragments, but not the 63-66 kDa group of α -fragments obtained by trypsin cleavage of the α -subunits molecules.³

In eukaryotic cells, clathrin-coated vesicles mediate the selective internalization of cell-surface receptors for lysosomal enzymes from the trans-Golgi network to a pre-lysosomal 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.⁶ 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* adaptor 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, Φ_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 (α_1 , α_2 , α_c1 and α_c2) 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 γ 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- α -Adaptin (AP-2) may be used for the localization of α -adaptin in immunoblotting.

Reagents

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

Precautions

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.

Product Profile

A minimum working dilution of 1:200 was determined by indirect immunoblotting using a crude preparation of synaptic vesicles obtained from rat cerebral cortex.

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

Storage

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.

References

1. Ahle, S., et al., EMBO J., **7**, 919 (1988).
2. Ahle, S., and Ungewickell, E., J. Cell Biol., **111**, 19 (1990).
3. Schroder, S., and Ungewickell, E., J. Biol. Chem., **266**, 7910 (1991).
4. Lindner, R., and Ungewickell, E., Biochemistry, **30**, 9097 (1991).
5. Lindner, R., and Ungewickell, E., J. Biol. Chem., **267**, 16567 (1992).
6. Robinson, M., Trends Cell Biol., **2**, 293 (1992).

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