3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-ARNO (CYTOHESIN-2)
CLONE CYT2-21
Purified Mouse Immunoglobulin

Product Number **A 4721**

Product Description

Monoclonal Anti-ARNO (Cytohesin-2) (mouse IgG1 isotype) is derived from the CYT2-21 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant human ARNO. The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2).

Monoclonal Anti-ARNO (Cytohesin-2) reacts specifically with ARNO, and does not cross-react with other members of the cytohesin family. The product is useful in ELISA, immunoblotting (apparent molecular weight of approximately 40 kDa), and immunocytochemistry. Reactivity has been observed with human and rat ARNO.

ARFs (ADP ribosylation factors) are GTP-binding proteins (20 kDa), which catalyze ADP-ribosylation of the α-subunit of the adenylyl cyclase-stimulatory G protein. ARFs have been shown to regulate various aspects of vesicular trafficking pathways in mammalian cells, including endocytosis, phagocytosis, secretion, and endoplasmic reticulum protein transport and budding of transport vesicles from Golgi in both anterograde and retrograde directions. Mammalian ARFs are grouped into three classes (class I: ARFs 1, 2, and 3, class II: ARFs 4 and 5, and class III: ARF 6), based on size, gene structure, and sequence identity.

ARFs are active when GTP, but not GDP or ATP, is bound. Hydrolysis of bound GTP to GDP with assistance of GTPase-activating protein, results in inactive ARF-GDP. Conversion of ARF-GDP to ARF-GTP is promoted by GEP (guanine-exchange protein).[1]

The ARF-GEP family of guanine nucleotide-exchange proteins (also referred to as cytohesins) includes cytohesin-1, ARNO (ARF nucleotide binding site opener, also called cytohesin-2 or Sec7p), and GRP1 (general receptor for phosphoinositides-1, also known as cytohesin-3 or ARNO3).

ARF-GEP family members are characterized by an N-terminal coiled-coil domain of 40 amino acids, a PtdIns(3,4,5)P_3-binding C-terminal PH (pleckstrin homology) domain, and a central Sec7 domain. Sec7 is a conserved catalytic domain of approximately 200 amino acids, which stimulates the exchange of GDP to GTP on members of the ARF family of GTPases.[2] The PH domain, by interacting with phospholipids, is believed to be responsible for association of cytoplasm with membranes.

ARNO (47 kDa) appears as a dimer of approximately 90 kDa in gel filteration, but has an apparent molecular weight of approximately 40 kDa in SDS-PAGE. It is 83% homologous to cytohesin-1.[3] However, while cytohesin-1 expression appears to be limited to hematopoietic cells, ARNO is more ubiquitously expressed.[4] The catalytic activity of ARNO has been localized to the region of the Sec7 domain, and it appears to be positively regulated by interaction of the PH domain with inositol phospholipids.[1] ARNO is localized to the plasma membrane in most mammlian cells, where it functions as an exchange factor for the plasma membrane-located ARF6 rather than ARF1.[5, 6] In HeLa cells, ARNO is mostly cytosolic, [7] and its recruitment from cytosol to endosomes in the epithelial cells of the kidney, is pH-dependent.[8]

Monoclonal antibody reacting specifically with ARNO (cytohesin-2) is a useful tool to study the role of ARNO in the regulation of vesicular trafficking pathways.

Reagent

Monoclonal Anti-ARNO (Cytohesin-2) is supplied as an approximately 2 mg/ml solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the 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 hazardous 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 is not recommended. Storage in "frost-free" freezers is also 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
A working concentration of 5-10 µg/ml is determined by immunoblotting, using a whole extract of rat brain.

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

References

1. Béraud-Dufour, S., et al., EMBO J., **17**, 3651-3659 (1998).
2. Pacheco-Rodriguez, G., et al., J. Biol. Chem., **273**, 26543-26548 (1998).
3. Chardin, P., et al., Nature, **384**, 481-484 (1996).
4. Kolanus, W., et al., Cell, **86**, 233-242 (1996).
5. Frank, S., et al., J. Biol. Chem., **273**, 23-27 (1998).
6. Venkateswarlu, K., et al., Curr. Biol., **8**, 463-466 (1998).
7. Monier, S., et al., J. Cell Sci., **111**, 3427-3436 (1998).
8. Maranda, B., et al., J. Biol. Chem., **276**, 18540-18550 (2001).

ZL/KAA 12/01