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ProductInformation

MONOCLONAL ANTI-GRP1 (CYTOHESIN-3, ARNO3)
CLONE CYT3-10
Purified Mouse Immunoglobulin

Product Number **G6541**

Product Description
Monoclonal Anti-GRP1 (Cytohesin-3, ARNO3) (mouse IgG1 isotype) is derived from the CYT3-10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a recombinant human GRP1. 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-GRP1 (Cytohesin-3, ARNO3) reacts specifically with GRP1, and does not cross-react with other members of the cytohesin family. The product is useful in ELISA, immunoblotting (45 kDa, and additional bands at 40 and 35 kDa), and immunocytochemistry. Reactivity has been observed with human and rat GRP1.

ARFs (ADP ribosylation factors) are GTP-binding proteins (20 kDa), which catalyze ADP-ribosylation of the α-subunit of the adenyl cyclase-stimulatory G protein. 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] 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. The ARF-GEP family, referred to as “cytohesins”, include cytohesin-1, ARNO (ARF nucleotide binding site opener, also called cytohesin-2 and Sec7p), and GRP1 (general receptor for phosphoinositides-1, also known as cytohesin-3 or ARNO3). The members of this family 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. It has been suggested that group I PH domains should be subdivided into group I_A, comprising those that specifically bind PtdIns(3,4,5)P_3 (i.e. Btk, ARNO, GRP1, cytohesin-1 and GAP1^m), and group I_B which has the ability to bind IP_4, but can bind PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 with comparable affinities (i.e. GAP1^{IP4BP}).[3] Indeed, GRP1 PH domain is unique among these proteins in its striking selectivity for PtdIns(3,4,5)P_3 versus PtdIns(4,5)P_2, for which it exhibits about 650-fold lower apparent affinity.[4] Receptor-mediated activation of phosphoinositide 3-kinase (PI 3-kinase) represents a major pathway by which biological systems operate to regulate diverse cellular functions, including vesicle transport, membrane ruffling and motility.[5] PtdIns(3,4,5)P_3 is a second messenger, generated by PI 3-kinase activation. GRP1 is involved in the control of Golgi structure and function, which is being mediated by the activation of ARF1 on Golgi membrane.[6] A physiological role for GRP1 in regulating ARF6 functions, has also been reported.[7]

Monoclonal antibody reacting specifically with GRP1 (Cytohesin-3, ARNO3) is a useful tool to study the role of GRP1 in the regulation of vesicular trafficking pathways.

Reagent
Monoclonal Anti-GRP1 (Cytohesin-3, Arno3) 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 2-4 µg/ml is determined by immunoblotting, using a whole extract of NRK (normal rat kidney) cells.

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. Cozier, G.E., et al., J. Biol. Chem., **275**, 28261-28268 (2000).
4. Klarlund, J.K., et al., J. Biol. Chem., **275**, 32816-32821 (2000).
5. Rameh, L.E., and Cantley, L.C., J. Biol. Chem., **274**, 8347-8350 (1999).
6. Franco, M., et al., Proc. Natl. Acad. Sci. USA, **95**, 9926-9931 (1998).
7. Langille, S.E., et al., J. Biol. Chem., **274**, 27099-27104 (1999).

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