

## Product Information

### MONOCLONAL ANTI-CYTOHESIN-1 CLONE CYT1-82

Purified Mouse Immunoglobulin

Product Number **C 8979**

#### Product Description

Monoclonal Anti-Cytohesin-1 (mouse IgG1 isotype) is derived from the CYT1-82 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a recombinant human cytohesin-1. The isotype is determined using ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Cytohesin-1 reacts specifically with cytohesin-1, and does not cross-react with other members of the cytohesin family. The product is useful in ELISA, immunoblotting (approximately 50 kDa, and possibly also additional bands at approximately 30 and 90 kDa), and immunocytochemistry. Reactivity has been observed with human cytohesin-1.

ARFs (ADP ribosylation factors) are GTP-binding proteins (20 kDa), which catalyze ADP-ribosylation of the  $\alpha$ -subunit of the adenylyl 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). Inhibition of GEP activity by brefeldin A (BFA), a fungal metabolite that reversibly causes apparent disintegration of Golgi in cells, has been reported.<sup>1</sup> 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 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 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<sub>3</sub>-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.<sup>2</sup> The PH domain, by interacting with phospholipids, is believed to be responsible for association of cytoplasm with membranes.

Cytohesin-1, the protein product of the *B2-1* gene, is a 47 kDa protein. It is abundant in cells of the immune system and is expressed at high levels in natural killer cells and peripheral T lymphocytes, and also expressed at very low levels in purified monocytes and several cultured cell lines. Cytohesin-1 contains two structural motifs: a large central Sec7 domain (200 amino acids) and a carboxy-terminal PH domain (100 amino acids). Overexpression of a cytohesin-1 Sec7 domain fusion protein in Jurkat cells markedly increases cell binding to the extracellular domain of ICAM-1, and direct interaction of the Sec7 domain with the cytoplasmic domain of  $\beta$ 1 integrin was demonstrated *in vitro*.<sup>3</sup> Cytohesin-1 acts as a GEP that accelerates GTP(yS) and GDP binding to purified native ARF3, and induces ARF binding to Golgi membranes via a mechanism insensitive to BFA.<sup>4</sup> Via its PH domain, cytohesin-1 can bind the lipid product of PI3-Kinase activation, a binding that is visualized through the recruitment of cytohesin-1 to the plasma membrane.<sup>5</sup> Also, granulocytic differentiation of HL-60 cells induced by cAMP results in a marked increase of the levels of endogenous cytohesin-1.<sup>6</sup>

Monoclonal antibody reacting specifically with cytohesin-1 is a useful tool for the study of the role of cytohesin-1 in the regulation of vesicular trafficking pathways.

#### **Reagent**

Monoclonal anti-Cytohesin-1 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 cultured human lymphoma Raji cells.

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

#### **References**

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2. Pacheco-Rodriguez, G., et al., *J. Biol. Chem.*, **273**, 26543-26548 (1998).
3. Kolanus, W., et al., *Cell*, **86**, 233-242 (1996).
4. Meacci, E., et al., *Proc. Natl. Acad. Sci. USA*, **94**, 1745-1748 (1997).
5. Venkateswarlu, K., et al., *J. Cell Sci.*, **112**, 1957-1965 (1999).
6. Garceau, V., et al., *J. Immunol. Meth.*, **249**, 121-136 (2001).

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