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Product Information Sheet

Rat Monoclonal Anti-human ß1 Integrin Antibody C27

PRODUCT INFORMATION

Catalog Number:	MABS1010
Hybridoma Clone:	C27
Lot Number:	C-001
Quantity:	0.1 mg
Concentration:	1.0 mg/mL
Antibody Type:	Rat IgG2a
Formulation:	0.1 mg antibody in protein-free hybridoma medium, PBS, pH 7.4, and 50% glycerol.
Storage:	- 20° C
Specificity:	Human ß1 integrin (also known as CD29, FNRB, MDF2, VLAB, GPIIA and MSK12)
Immunogen:	ß1 integrin isolated from LOX human melanoma cells
Applications:	Immunocytochemistry, Immunoprecipitation, Western blotting, Flow cytometry, ELISA, Inhibition of cell adhesion to ECM substrata and Functional blocker of cell migration.
Method of protein determination:	SDS-PAGE analysis showing greater than 99% protein being IgG heavy chain at 55-kDa and light chain at 25-kDa and Bradford method.
Method of activity determination:	Western immunoblotting, immunofluorescence and inhibition of integrin function.

DESCRIPTION

This antibody is produced from the C27 hybridoma cell line derived from fusion of rat myeloma Y3 cells and spleen cells of a Sprague-Dawley rat that was immunized with glycoproteins isolated from melanoma LOX cell membranes (Mueller et al., 1999). Further study confirmed that this antibody targets human ß1 integrin. Integrins are heterodimeric proteins composed of an alpha and a beta subunit. Integrin family members are membrane receptors involved in cell adhesion and recognition in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response and metatastatic diffusion of tumor cells. ß1 integrin, also known as CD29, FNRB, MDF2, VLAB, GPIIA and MSK12 [Gene ID: 3688; Accession#: NP_002202], is a beta subunit. Six alternatively spliced variants have been found that encode five proteins with alternate carboxyl termini.

PREPARATION

MAb C27 was produced by C27 hybridoma cells in protein-free medium using CELLine CL 1000 Disposable Bioreactors (INTEGRA Biosciences) that includes a 10 kDa semi-permeable cellulose acetate membrane to exclude small molecules. Supernatant from the cell compartment was cleared by spinning at 10,000rpm. Approximate 5 mg/mL of antibody was obtained and diluted to 2.0 mg/mL using PBS, pH 7.4. Equal volume of glycerol was added to the antibody solution to make the final concentration of 1.0 mg/mL.

METHOD OF ACTIVITY DETERMINATION

The activity of the antibody was determined using Western immunoblotting, immunofluorescence and adhesion inhibition assays (see Specificity below).

METHOD OF PROTEIN DETERMINATION

Protein concentration was determined using SDS-PAGE under reducing and denaturing conditions. Antibody protein was determined for its identity by SDS-PAGE analysis that shows greater than 99% of total protein being IgG heavy chain at 55-kDa and light chain at 25-kDa. The total protein in the preparation was measured with Bradford protein assay using Quick Start Bradford Dye Reagent (Bio-Rad); serially diluted BSA samples were used as standards.

STORAGE

The antibody may be stored at -20° C for one year in its original formulation. Additionally, antibody diluted with 1% BSA in PBS may be stored at 2° to 8° C for up to 1 month without detectable loss of activity. **Avoid repeated freeze-thaw cycles of the diluted antibody.**

SPECIFICTY

MAb C27 recognizes human ß1 integrin. In Western blotting, it does not react with ß3 integrin (Mueller et al., 1999). This antibody is inhibitory to binding/attachment of cells to collagenous substrata (Ghersi et al., 2002; Ghersi et al., 2006); it also blocks the migration

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and local matrix degradation of endothelial cells in collageneous matrices (Ghersi et al., 2006). Furthermore, this antibody can block the formation of cellular networks by endothelial cells in Matrigel (Ghersi et al., 2006).

APPLICATIONS

Immunocytochemistry and Immunofluorescence – This antibody is also effective for direct immunofluorescence staining of human ß1 integrin in cells (Ghersi et al., 2002).

Immunoprecipitation – This antibody has been used to immuno-precipitate human ß1 integrin from cell lysates (Mueller et al., 1999).

Inhibition of integrin function – This antibody has been used to block ß1 integrin mediated cell adhesion to fibronectin, laminin, collagen, and gelatin substrata (Mueller et al., 1999; Ghersi et al., 2006). This antibody also blocks the migration and local matrix degradation of endothelial cells in collageneous matrices (Ghersi et al., 2006). Furthermore, this antibody can block the formation of cellular networks by endothelial cells in Matrigel (Ghersi et al., 2006).

Western Blotting – MAb C27 can be used with the appropriate secondary reagents to detect ß1 integrin in Western blotting (Mueller et al., 1999; Ghersi et al., 2006).

REFERENCES

Ghersi,G., Dong,H., Goldstein,L.A., Yeh,Y., Hakkinen,L., Larjava,H.S., and Chen,W.-T. (2002). Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. J. Biol. Chem. *277*, 29231-29241.

Ghersi,G., Zhao,Q., Salamone,M., Yeh,Y., Zucker,S., and Chen,W.-T. (2006). The protease complex consisting of dipeptidyl peptidase IV and seprase plays a role in the migration and invasion of human endothelial cells in collagenous matrices. Cancer Res *66*, 4652-4661.

Mueller,S.C., Ghersi,G., Akiyama,S.K., Sang,Q.X., Howard,L., Pineiro-Sanchez,M., Nakahara,H., Yeh,Y., and Chen,W.-T. (1999). A novel protease-docking function of integrin at invadopodia. J. Biol. Chem. *274*, 24947-24952.

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