

Rat Monoclonal Anti-human CD44 Antibody C37

PRODUCT INFORMATION

Catalog Number:	MABS1011
Hybridoma Clone:	C37
Lot Number:	D-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 CD44 antigen
Immunogen:	CD44 isolated from LOX human melanoma cells
Applications:	Immunocytochemistry, Immunoprecipitation, Flow cytometry, ELISA and Western blotting.
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 and immunofluorescence.

DESCRIPTION

This antibody is produced from the C37 hybridoma cell line derived from fusion of rat myeloma Y3 cells and spleen cells of an immunized Sprague-Dawley rat. The immunogen was a p90 glycoprotein that was co-isolated with seprase from melanoma cell membranes (Monsky et al., 1994). Further study confirmed that the p90 glycoprotein is human CD44 antigen [Gene ID: 960; Accession#: NP_000601.3], which is also known as IN, LHR, MC56, MDU2, MDU3, MIC4, Pgp1, CDW44, HCELL, MUTCH-I, ECMR-III and MGC10468. CD 44 antigen is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. It is a receptor for hyaluronic acid (HA) and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). This protein participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis. Alternative splicing is the basis for the structural and functional diversity of this protein, and may be related to tumor metastasis.

PREPARATION

MAb C37 was produced by C37 hybridoma cells in protein-free medium using CELLline 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 and immunofluorescence (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.**

SPECIFICITY

MAb C37 recognizes human CD44 antigen. This antibody does not block the migration and invasion of fibroblasts and endothelial cells on collagenous matrices (Gherzi et al., 2002; Gherzi et al., 2006); neither it blocks the formation of the cellular networks by endothelial cells in Matrigel (Gherzi et al., 2006).

APPLICATIONS

Immunocytochemistry and Immunofluorescence – This antibody is also effective for direct immunofluorescence staining of human CD44 on cell surfaces (Chen and Wang, 1999; Gherzi et al., 2006).

Immunoprecipitation – This antibody has been used to immuno-precipitate human CD44 antigen from cell lysates (Monsky et al., 1994).

Flow Cytometry- This antibody has been used in cell sorting (Monsky et al., 1994).

Western Blotting- MAb C37 can be used with the appropriate secondary reagents to detect human CD44 antigen in Western blotting (Monsky et al., 1994).

REFERENCES

Chen, W.-T. and Wang, J.Y. (1999). Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2, and TIMP-2 localization. [Review] [52 refs]. *Annals of the New York Academy of Sciences* 878, 361-371.

Gherzi, 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.

Gherzi, 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.

Monsky, W.L., Lin, C.-Y., Aoyama, A., Kelly, T., Mueller, S.C., Akiyama, S.K., and Chen, W.-T. (1994). A potential marker protease of invasiveness, seprase, is localized on invadopodia of human malignant melanoma cells. *Cancer Res.* 54, 5702-5710.

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