

Rat Monoclonal Anti-human Seprase Antibody E97

PRODUCT INFORMATION

Catalog Number: MABS1004
Hybridoma Clone: E97
Lot Number: A-004
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 seprase (also called fibroblast activation protein alpha, FAP α)
Immunogen: Dissociated human seprase subunits
Applications: Immunohistochemistry 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 using recombinant antigen produced by 293-EBNA human kidney cells.

DESCRIPTION

This antibody is produced from the E97 hybridoma cell line derived from fusion of rat myeloma Y3 cells and spleen cells of an immunized Sprague-Dawley rat (Pineiro-Sanchez et al., 1997). Seprase, also called fibroblast activation protein alpha (FAP α) [Gene ID: 2191; Accession#: NP_004451] is a 170-kDa homodimeric integral membrane gelatinase belonging to the type II transmembrane serine protease family. It has a non-classical serine catalytic site and exhibits dipeptidyl dipeptidase and gelatinase activities. Seprase is selectively expressed in various cell types from cancerous tissues as well as tissues in the early stages of wound healing (Chen et al., 2003). This protein is thought to be involved in tumor invasion, angiogenesis and metastasis as well as embryo development and tissue repair. Recently, truncated and active forms of seprase have been detected in human malignant tumors (Chen et al., 2006) and plasma (Lee et al., 2006).

PREPARATION

MAb E97 was produced by E97 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

Western immunoblotting (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 (Figure 1). 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

This antibody was developed using heat-denatured human placental seprase as an immunogen and screened by Western immunoblotting. In Western immunoblotting, this antibody recognizes the dissociated monomers of both full-length seprase and recombinant seprase excluding the cytoplasmic (amino acids 1-6) and transmembrane domain (amino acids 7-26); it does not react

with non-denatured, enzymatically active dimeric seprase forms (Chen et al., 2006). Furthermore, this antibody does not block the peptidase and gelatinase activity of seprase. It does not recognize mouse seprase in Western blotting (Chen et al., 2006).

APPLICATIONS

Immunohistochemistry – MAb E97 may be used to detect human seprase via immunohistochemistry techniques. Using an antibody concentration of 1 to 5 µg/mL, human seprase has been identified in paraffin-embedded tissue sections (Iwasa et al., 2005). Antigen retrieval may be achieved by heating in an autoclave in 0.01M citrate buffer (pH6.0).

Western Blotting-This antibody is excellent for human seprase detection using Western blotting (Chen et al., 2006; Iwasa et al., 2005; Okada et al., 2003). This antibody recognizes denatured seprase 97-kDa monomers and truncated seprase forms (Chen et al., 2006).

REFERENCES

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