

## Rat Monoclonal Anti-human Seprase Antibody D43

### PRODUCT INFORMATION

<b>Catalog Number:</b>	MABS1003
<b>Hybridoma Clone:</b>	D43
<b>Lot Number:</b>	A-003
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	1.0 mg/mL
<b>Antibody Type:</b>	Rat IgG2a
<b>Formulation:</b>	0.1 mg antibody in protein-free hybridoma medium, PBS, pH 7.4, and 50% glycerol.
<b>Storage:</b>	- 20° C
<b>Specificity:</b>	Human seprase (also called fibroblast activation protein alpha, FAP $\alpha$ )
<b>Immunogen:</b>	Seprase isolated from human termed placenta
<b>Applications:</b>	Immunohistochemistry, Immunocytochemistry, Immunofluorescence, Flow cytometry, ELISA, Immunocapture, Immunoprecipitation and Western blotting.
<b>Method of protein determination:</b>	SDS-PAGE analysis showing greater than 99% protein being IgG heavy chain at 55-kDa and light chain at 25-kDa and Bradford method.
<b>Method of activity determination:</b>	Immuno-capture of recombinant antigen produced by 293-EBNA human kidney cells.

### DESCRIPTION

This antibody is produced from the D43 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 $\alpha$ ) [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 D43 was produced by D43 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 an immunocapture assay. Briefly, anti-rat antibody was coated on a plate and 50  $\mu$ g/mL of target rat monoclonal antibody was added. Native seprase from human tumor cells or recombinant human seprase was then added. The prolyl dipeptidase and serine gelatinase activities of the captured seprase were measured using seprase substrates Gly-Pro-p-Nitroanilide (sigma) and DQ gelatin (Invitrogen), respectively. Alternatively, antigen captured by the target antibody was detected using biotinylated anti-seprase antibody D8, followed by a colorimetric assay.

### 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.



Figure 1. SDS-PAGE analysis of mAb D43 under denaturing and reducing conditions. 5  $\mu$ g/lane.

## 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 recognizes human full-length seprase and recombinant seprase excluding the cytoplasmic (amino acids 1-6) and transmembrane domains of seprase (amino acids 7-26). This antibody recognizes an epitope that is different from the binding sites of mAb D8, mAb D28 and mAb E97. In Western blotting, it does not recognize mouse seprase (Chen et al., 2006). Furthermore, this antibody does not block seprase peptidase activity against Gly-Pro-pNA (Gherzi et al., 2002; Gherzi et al., 2006); it does inhibit the gelatinase activity of seprase.

This antibody is specific for the dimeric, proteolytically active, form of seprase in ELISA, Immunocapture (Chen et al., 2006), Western Immunoblotting (Pineiro-Sanchez et al., 1997; Monsky et al., 1994), and Immuno-precipitation assays (Chen et al., 2006); it does not recognize dissociated seprase subunits.

## APPLICATIONS

**Immunohistochemistry** – MAb D43 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; Okada et al., 2003). Antigen retrieval may be achieved by heating in an autoclave in 0.01M citrate buffer (pH 6.0).

**Immunocytochemistry and Immunofluorescence** – This antibody is effective for direct and indirect immunofluorescence staining of human seprase at cell surfaces. Human seprase concentrated at cell protrusions of tumor cells was identified via fluorescence and confocal microscopy using an antibody concentration of 1 to 10 µg/mL (Pineiro-Sanchez et al., 1997).

**ELISA** – This antibody can be used in ELISA at a concentration of 5 to 50 µg/mL to detect purified seprase coated on plastic plates (Nakahara et al., 1996).

**Immuno-capture** – This antibody has been employed to capture native seprase and recombinant seprase in their active forms (Chen et al., 2006). In one assay performed (Figure 2), the antibody was linked to a solid support to capture seprase in cell lysates. The prolyl dipeptidase and serine gelatinase activities of seprase were measured using Gly-Pro-p-Nitroanilide (sigma) and DQ gelatin (Invitrogen), respectively.

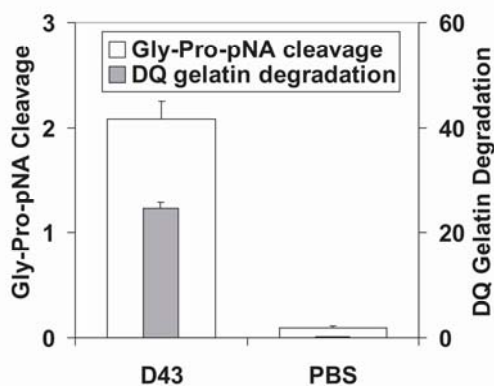


Figure 2. Immunocapture analysis of seprase in human melanoma LOX cells. Cell lysate was applied to plastic wells coated with mAb D43 and the captured seprase was detected by measuring its prolyl dipeptidase and serine gelatinase activities as described (Chen et al., 2006). PBS was used as a negative control for the cell lysate. The experiment was performed in triplicate and the values are the mean ±SD.

**Immunoprecipitation** – This antibody has been conjugated to agarose beads to precipitate human seprase dimer from tumor lysates (Chen et al., 2006), human placental tissue lysates (Pineiro-Sanchez et al., 1997) and human malignant melanoma LOX cells (Monsky et al., 1994)(Pineiro-Sanchez et al., 1997).

**Western Blotting** – This antibody can be used at 1 to 10 µg/mL with the appropriate secondary reagents to detect human seprase in Western blotting. This antibody recognizes seprase 170KD dimers but not the dissociated 97-kDa monomers (Pineiro-Sanchez et al., 1997).

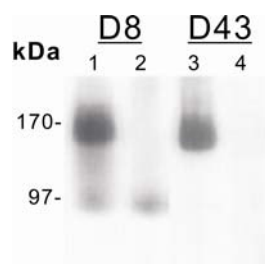


Figure 3. Western blotting analysis of seprase using mAbs D8 and D43. Seprase protein enriched from melanoma LOX cells was subjected to SDS-PAGE under non-denaturing (lanes 1 and 3) or denaturing and reducing (lanes 2 and 4) conditions, followed by immunoblotting using mAbs D8 or D43. MAb D43 recognizes dimeric seprase (170-kDa) but not the 97-kDa subunit (Pineiro-Sanchez et al., 1997).

**REFERENCES**

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