

## Rat Monoclonal Anti-human Seprase Antibody D8

### PRODUCT INFORMATION

<b>Catalog Number:</b>	MABS1001
<b>Hybridoma Clone:</b>	D8
<b>Lot Number:</b>	A-001
<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, Inhibition of seprase-gelatinase activity, 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 D8 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 D8 was produced by D8 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.

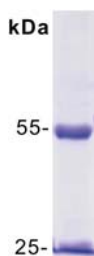


Figure 1. SDS-PAGE analysis of mAb D8 under denaturing and reducing conditions. 5  $\mu$ g/lane.

**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 D28, mAb D43 and mAb E97. In Western blotting, it does not recognize mouse seprase (Chen et al., 2006); in immunoprecipitation, it does not detect human DPPIV (Gherzi et al., 2006), a homolog of seprase. 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.

**APPLICATIONS**

**Immunohistochemistry** – MAb D8 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, for examples see (Chen et al., 2006; Gherzi et al., 2002; Gherzi et al., 2006; Iwasa et al., 2005; Mori et al., 2004; Okada et al., 2003). Antigen retrieval may be achieved by heating in an autoclave in 0.01M citrate buffer (pH6.0).

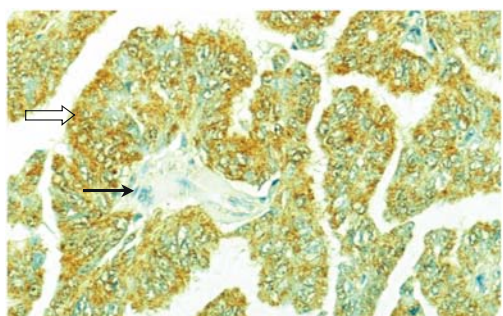


Figure 2. Immunohistochemistry of seprase in tumor cells (open arrow) but not stromal cells (arrow) on a paraffin section of malignant ovarian carcinoma using mAb D8 (cited from Figure 4C of Chen et al., 2006).

**Immunocytochemistry and Immunofluorescence** – This antibody is also effective for direct and indirect immunofluorescence staining of human seprase at cell surfaces. Human seprase concentrated at cell protrusions of tumor and endothelial cells as well as fibroblasts was identified via fluorescence and confocal microscopy using an antibody concentration of 1 to 10 µg/mL (Aoyama and Chen, 1990; Artym et al., 2002; Chen et al., 2006; Gherzi et al., 2002; Gherzi et al., 2006; Goldstein et al., 1997; Mueller et al., 1999; 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 96-well microtiter plates (Chen et al., 2006; Nakahara et al., 1996; Pineiro-Sanchez et al., 1997).

**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, the antibody is linked to a solid support to capture seprase in biological fluid. Then the prolyl dipeptidase and serine gelatinase activities of seprase are measured using seprase substrates Gly-Pro-p-Nitroanilide (sigma) and DQ gelatin (Invitrogen), respectively.

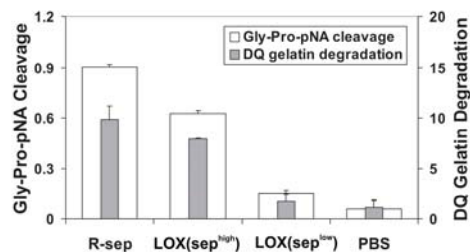


Figure 3. Immunocapture analysis of seprase proteolytic activities. Lysates of tumors derived from seprase-expressing LOX melanoma cells (sep<sup>high</sup>) and from seprase-suppressed cells (sep<sup>low</sup>) were applied to a 96-well microtiter plate coated with mAb D8 to capture seprase. Seprase enzymatic activities are then measured as described (Chen et al., 2006). Recombinant seprase (r-sep) and PBS were used as positive and negative controls, respectively. The experiment was performed in triplicate and the values are the mean ±SD.

**Immunoprecipitation** – This antibody has been conjugated to agarose beads to successfully immuno-precipitate human seprase from human tumor tissue lysates (Chen et al., 2006) and lysates from different cells in culture (Chen et al., 2006; Gherzi et al., 2002;

Gherzi et al., 2006; Goldstein et al., 1997; Mueller et al., 1999; Nakahara et al., 1996; Pineiro-Sanchez et al., 1997). This antibody may also work indirectly for immunoprecipitation by using anti-rat IgG antibody conjugated beads.

**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 (Chen et al., 2006; Gherzi et al., 2002; Gherzi et al., 2006; Goldstein et al., 1997; Goldstein and Chen, 2000; Iwasa et al., 2005; Mori et al., 2004; Mueller et al., 1999; Nakahara et al., 1996; Okada et al., 2003; Pineiro-Sanchez et al., 1997). This antibody recognizes both seprase 170-kDa dimers and the dissociated 97-kDa monomers.

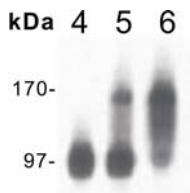


Figure 4. Western blotting analysis of seprase dissociation by treatment with acidic pH. Purified seprase from LOX melanoma cells was incubated with citric acid/phosphate buffers at pH 4, 5, and 6 for 10 min and then subjected to immunoblotting using mAb D8 as described (Pineiro-Sanchez et al., 1997). MAb D8 recognizes both seprase 170-kDa dimers and the 97-kDa subunit.

## REFERENCES

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