

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse MMP-7: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 704202

Isotype: rat IgG_{2B}

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

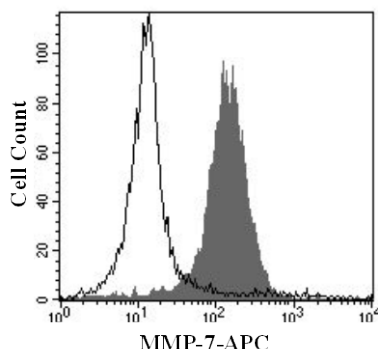
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing MMP-7 within a population and qualitatively determine the density of intracellular MMP-7 by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, NS0-derived, recombinant mouse matrix metalloproteinase-7 (rmMMP-7). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of MMP-7 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



bEND.3 cells treated with Recombinant Mouse FGF basic (Catalog # 3139-FB) were stained with APC-conjugated anti-mouse MMP-7 (Catalog # IC7170A, filled histogram) or APC-conjugated isotype control (Catalog # IC013A, open histogram).

Background Information

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP-7 (matrilysin) is expressed in epithelial cells of normal and diseased tissues and is capable of digesting a large series of proteins of the extracellular matrix including collagen IV and X, gelatin, casein, laminin, aggrecan, entactin, elastin, and versican.¹ MMP-7 is implicated in the activation of other proteinases such as plasminogen, MMP-1, MMP-2, and MMP-9. In addition to its roles in connective tissue remodeling and cancer, MMP-7 also regulates intestinal α -defensin activation in innate host defense, releases tumor necrosis factor- α in a model of herniated disc resorption, and cleaves FasL to generate a soluble form in a model of prostate involution. Structurally, MMP-7 is the smallest of the MMPs and consists of two domains: a prodomain that is cleaved upon activation and a catalytic domain containing the zinc-binding site.

Reference

1. Woessner, J.F. (2004) in Handbook of Proteolytic Enzymes, Barrett, A.J. et al. eds. p. 532.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Resuspend up to 1×10^6 cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 µL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled rat IgG_{2B} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.