

MONOCLONAL ANTIBODY

# Anti-MFG-E8 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D199-3	18A2-G10	Hamster IgG	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Apoptotic cells are rapidly removed by phagocytosis to clear the living cells and tissues of intracellular materials being released from dying cells. Phagocytes recognize apoptotic cells through milk fat globule-EGF-factor 8 (MFG-E8). MFG-E8 is a secreted glycoprotein from thioglycollate-elicited macrophages and binds to phosphatidylserine exposed on the cell surface of apoptotic cells, then binds strongly to cells expressing  $\alpha$ v $\beta$ 3 integrin via its RGD (arginine-glycine-aspartate) motif. Thus, MFG-E8 is an important protein to mediate the engulfment of apoptotic cells by activated macrophages.

**SOURCE:** This antibody was purified from hybridoma (clone 18A2-G10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell with Armenian hamster lymph node immunized with the recombinant mouse MFG-E8.

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at -20°C.

**REACTIVITY:** This antibody reacts with mouse MFG-E8 short and long forms on Western blotting and Immunohistochemistry.

## APPLICATIONS:

Western blotting: 1  $\mu$ g/mL for chemiluminescence detection system

Immunoprecipitation: Not recommended\*

\*D161-3 (clone 2422) is suitable for this application.

Immunohistochemistry: 1  $\mu$ g/mL (frozen sections)

Immunocytochemistry: Not tested

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOLS.**

## SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell, Tissue	Not Tested	transfectant, epididymides	Not Tested
Reactivity on WB		+	

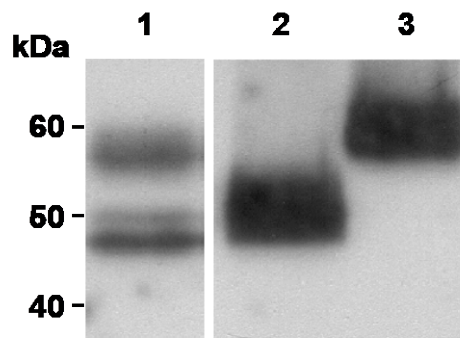
## INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

## REFERENCES:

- 1) Middendorp, S., *et al.*, *Blood*. **114**, 2280-9 (2009) [IHC]
- 2) Kranich, J., *et al.*, *J. Exp. Med.* **205**, 1293-1302 (2008) [IHC]
- 3) Aziz, M. M. *et al.*, *Apoptosis*. **13**, 609-20 (2008) [WB]
- 4) Yoshino, M. *et al.*, *Immunol. Lett.* **115**, 131-137 (2008) [IHC]
- 5) Jinushi, M., *et al.*, *J. Clin. Invest.* **117**, 1902-1913 (2007) [IHC]
- 6) Hanayama, R., *et al.*, *PNAS* **102**, 16886-16891 (2005)
- 7) Hanayama, R., *et al.*, *Science* **304**, 1147-1150 (2004)
- 8) Miyasaka, K., *et al.*, *Eur. J. Immunol.* **34**, 1414-1422 (2004)
- 9) Hanayama, R., *et al.*, *J. Immunol.* **172**, 3876-3882 (2004)
- 10) Hanayama, R., *et al.*, *Nature* **417**, 182-187 (2002)

Clone 18A2-G10 is used in reference number 1) - 8).



**Western blot analysis of MFG-E8 expression on mouse epididymides (1), recombinant HPC4 tagged mouse MFG-E8 short form (2) and long form (3) using D199-3.**

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

## PROTOCOLS:

### SDS-PAGE & Western Blotting

- 1) Epididymides from 10 mice (10 to 14 weeks of age) were homogenized with a Dounce homogenizer in 10 mL of the ice-cold buffer (125 mM NaCl, 25 mM MOPS pH 7.5) containing appropriate protease inhibitors.
- 2) Centrifuge the crude homogenate at 800 x g for 10 minutes at 4°C and transfer the supernatant to another tube, then subjected to a high-speed centrifugation (100,000 x g) for 60 minutes at 4°C.
- 3) Remove the supernatant, and then resuspend the pellet in 4 mL of the following buffer (125 mM NaCl, 25 mM TAPS pH 9.0, 1% Triton X-100).

- 4) Mix the sample with equal volume of Laemmli's sample buffer.
- 5) Boil the samples for 3 minutes and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 8) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 10) Incubate the membrane with the 1:1,000 HRP-conjugated anti-hamster IgG (Santa Cruz; code no. sc-2443) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 11) Wash the membrane with PBS-T (10 minutes x 3 times).
- 12) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 13) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 14) Expose to an X-ray film in a dark room for 3 minutes.
- 15) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; transfectant, mouse epididymides)

#### **Immunohistochemical staining for frozen sections:**

##### **SAB method**

- 1) The mouse epidermal tissues are fixed in acetone for 15 minutes.
- 2) Immerse sections in PBS for 10 minutes at room temperature.
- 3) Cover each section with 3% H<sub>2</sub>O<sub>2</sub> in MeOH for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
- 4) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 30 minutes to block non-specific staining. Do not wash.
- 5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
- 6) Incubate the sections for 1 hour at room temperature.
- 7) Wash the slides 3 times in PBS for 5 minutes each.
- 8) Wipe gently around each section and cover tissues with Biotin labeled anti-hamster IgG (Santa Cruz; code no.

sc-2445). Incubate for 10 minutes at room temperature. Wash as in step 7).

- 9) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 7).
- 10) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40  $\mu$ L of 30% H<sub>2</sub>O<sub>2</sub> in 150 mL PBS. \*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 11) Wash the slides in water for 5 minutes.
- 12) Now ready for mounting.

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