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

MONOCLONAL ANTIBODY

Anti-MFG-E8 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D199-3	18A2-G10	Hamster IgG	100 µL	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 µg IgG in 100 µL 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 µg/mL
Immunoprecipitation; Not recommended*
*D161-3 (clone 2422) is suitable for this application.
Immunohistochemistry; 1 µg/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:

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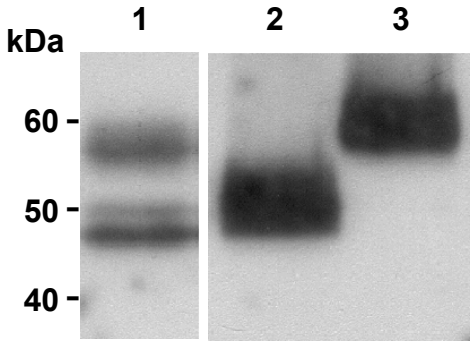
REFERENCES:

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 µL 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^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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_2O_2 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 suggested 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 µL of 30% H_2O_2 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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