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

MBL

MONOCLONAL ANTIBODY

Anti-MFG-E8 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D161-3	2422	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 bind to phosphatidylserine exposed on the cell surface of apoptotic cells, then bind strongly to cells expressing αvβ3 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 2422) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell with Armenian hamster lymph node immunized with thioglycollate-elicited mouse peritoneal macrophages.

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 form on Flow cytometry and Immunoprecipitation.

APPLICATIONS:

Western blotting; Not recommended*
*[MBL, code no. D199-3] is suitable for this application.
Immunoprecipitation; 5 µg/250 µL of mouse bone marrow cells culture supernatant
Immunohistochemistry; Not tested*
*It is reported that this monoclonal antibody can be used in Immunohistochemistry in the reference number 6).
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)
ELISA; Not tested*
*It is reported that this monoclonal antibody can be used in ELISA in the reference number 8).
Neutralization; Not tested*
*It is reported that this monoclonal antibody can be used in Neutralization in the reference number 1).

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissues and other	Not tested	recombinant, bone marrow, epididymides	Not tested
Reactivity on IP		+	

REFERENCES:

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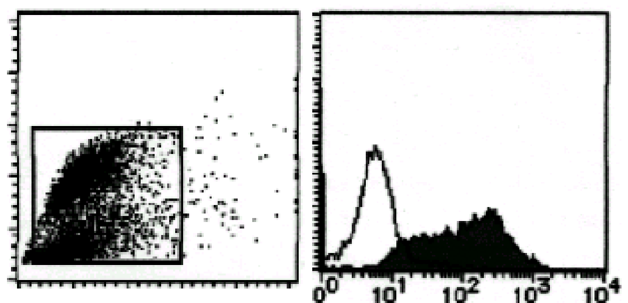
2) Zhang, F., *et al., J. Trauma Acute Care Surg.* **72**, 861-869 (2012) [WB]
3) Usui, K., *et al., Mol. Immunol.* **50**, 172-176 (2012) [FCM]
4) Cui, T., *et al., Am. J. Respir. Crit. Care Med.* **181**, 238-246 (2010) [WB]
5) Hoffhines, A. J., *et al., J. Biol. Chem.* **284**, 3096-3105 (2009) [IP]
6) Fens, M. H, *et al., Blood* **111**, 4542-4550 (2008) [IHC]
7) Montecalvo, A., *et al., J. Immunol.* **180**, 3081-3090 (2008)
8) Miksa, M., *et al., Mol. Med.* **13**, 553-560 (2007)[ELISA]
9) Hanayama, R., *et al., Science* **304**, 1147-1150 (2004)
10) Morelli, A. E., *et al., Blood* **104**, 3257-3266 (2004)
11) Hanayama, R., *et al. J. Immunol.* **172**, 3876-3882 (2004)
12) Hanayama, R., *et al. Nature* **417**, 182-187 (2002)

Clone 2422 is used in these references.



Flow cytometric analysis of MFG-E8 binding to apoptotic Jurkat cells. *Apoptotic Jurkat cells induced by anti-Fas (CH-11/code no. SY-001) were incubated with recombinant MFG-E8. MFG-E8 bound to the apoptotic cells was detected with D161-3. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D161-3 to the cells.*

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

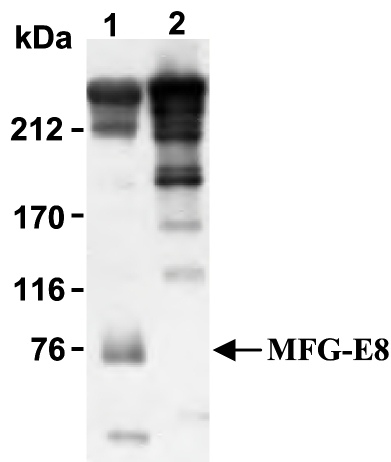
PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Induce apoptosis to Jurkat cells by Anti-Fas (CD95) mAb (clone CH-11, MBL code no. SY-001).
2) Incubate the apoptotic cells with 400 µL of 0.25 µg/mL recombinant MFG-E8 diluted with PBS containing 2% FCS for 30 minutes at room temperature (20~25°C).
3) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
4) Resuspend the cells with washing buffer (5×10^6 cells/mL).
5) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 20 µL of 10 µg/mL of PE conjugated anti-hamster IgG (BD Pharmingen; code no. 554056) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Apoptotic Jurkat)



Immunoprecipitation of MFG-E8 from culture supernatant of mouse bone marrow cells with D161-3 (1) or hamster IgG (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D199-3.*

Immunoprecipitation

1) Collect culture supernatant of mouse bone marrow cells into a culture tube.
2) Centrifuge the tube at 400 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40]. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
6) Resuspend the beads with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
8) Repeat steps 6)-7) 3-5 times
9) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
10) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
11) 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% methanol). See the manufacture's manual for precise transfer procedure.
12) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
13) Incubate the membrane with 1 µg/mL of Anti-MFG-E8 (Mouse) mAb (clone 18A2-G10, MBL code no. D199-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
15) Incubate the membrane with the HRP-conjugated anti-hamster IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
16) Wash the membrane with PBS-T (5 minutes x 3 times).
17) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; culture supernatant of mouse bone marrow)

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