D161-3 Page 1 of 2	For Resear Not for use	ch Use Only. in diagnostic pr	ocedures.	A JSR Life Sciences Company
MONOCLON	AL ANTIBODY			
	Anti-MF	G-E8 (Mo	ouse) ma	Ab
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 $\alpha\nu\beta3$ 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:

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

SPECIES CROSS REACTIVITY:

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

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- 5) Hoffhines, A. J., et al., J. Biol. Chem. 284, 3096-3105 (2009) [IP]
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- 7) Montecalvo, A., et al., J. Immunol. 180, 3081-3090 (2008)
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- 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)
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Clone 2422 is used in these references.



analysis MFG-E8 Flow cytometric of 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.

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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).
- 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% NaN₃].
- 4) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
- 5) Add 100 μ L 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 μ L 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 μ L 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 μ L 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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 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/cm² 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 μ g/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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