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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-EGF-R (Human) mAb

Code No.	Clone	Subclass	Quantity
MI-12-1	6F1	Mouse IgG2b κ	100 µL

BACKGROUND: The epidermal growth factor receptor (EGFR, ErbB-1) is a 170 kDa transmembrane glycoprotein possessing intrinsic tyrosine kinase activity. There are several EGF family ligands including epidermal growth factor (EGF), amphiregulin (AR), heparin-binding epidermal growth factor (HB-EGF), epiregulin, betacellulin, transforming growth factor-α (TGF-α) and epigen, which can bind and activate the EGFR. Ligand binding facilitates dimerization of the EGFR, which activates downstream pathways known to be involved in cell growth, proliferation, differentiation and migration.

Other members of the receptor tyrosine kinase family, which include EGFR, ErbB-2/HER-2, ErbB-3/HER-3 and ErbB-4/HER-4, are also known to play important roles in regulating a wide variety of cellular functions. Overexpression by gene amplification or mutation of these receptors correlates with the development and progression of several human cancers, as well as poor prognosis. Consequently, these receptors have been identified not only as a possible prognostic markers but also primarily a rational molecular target for an anticancer agents.

SOURCE: This antibody was concentrated from hybridoma (clone 6F1) supernatant. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human EGF-R cytoplasmic region (648-1186 aa).

FORMULATION: The protein concentration should be 1 mg/mL. And the IgG concentration should be 100 µg/mL in 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 human EGF-R on Western blotting, Immunoprecipitation, Immunocytochemistry and Flow cytometry. This antibody does not recognize human c-erbB2.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	A431	Not tested	Not tested
Reactivity on WB	+		

INTENDED USE:
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APPLICATIONS:
Western blotting; 1:1,000-1:2,000
Immunoprecipitation; 2-5 µL/250 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; This antibody is not available for paraffin embedded sections.
Immunocytochemistry; 1:100
Flow cytometry; 1:100 (final concentration)

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

REFERENCES:
1) Ishikawa, K., *et al., Mol. Biol. Cell* **23**, 1294-1306 (2012)
2) Yamasaki, A., *et al., Mol. Biol. Cell* **17**, 4876-4887 (2006) [WB]
3) Mizuno, E., *et al., Mol. Biol. Cell* **16**, 5163-5174 (2005) [WB][IP][IC]
4) Mizuno, E., *et al., Mol. Biol. Cell* **14**, 3675-3689 (2003) [IC]
5) Nakamura, T., *et al., Mol. Cell Biol.* **22**, 8721-8734 (2002)[IC]
6) Liu, X. W., *et al., J. Biol. Chem.* **276**, 5068-5073 (2001) [WB][IC]
7) Lazarovici, P., *et al., Mol. Pharmacol.* **54**, 547-558 (1998) [WB]
8) Hashimoto, Y., *et al., J. Biol. Chem.* **273**, 17186-17191 (1998) [IP]
9) Shibutani, M., *et al., J. Biol. Chem.* **273**, 6878-6884 (1998) [WB]
10) Ichiba, T., *et al., J. Biol. Chem.* **272**, 22215-22220 (1997) [WB]
11) Lazarovici, P., *et al., J. Biol. Chem.* **272**, 11026-11034 (1997)

Clone 6F1 is used in these references.

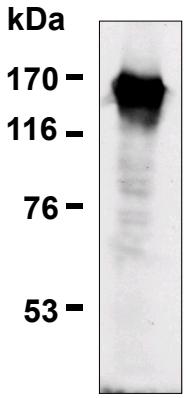
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) 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.
4) 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 4ºC.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 anti-IgG (H+L) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; A431)

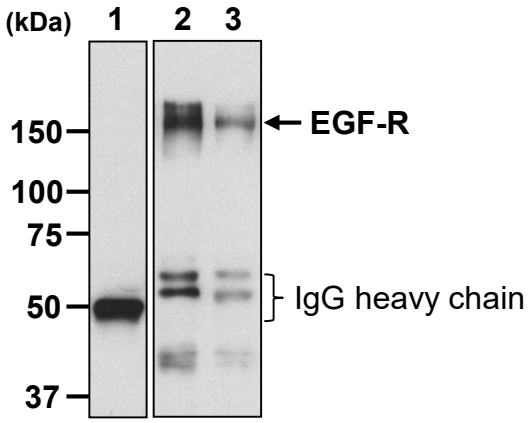


Western blotting analysis of human EGF-R expression in A431 using MI-12-1.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4ºC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4ºC and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 100 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4ºC.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4ºC.
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 beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; A431)



Immunoprecipitation of EGF-R protein

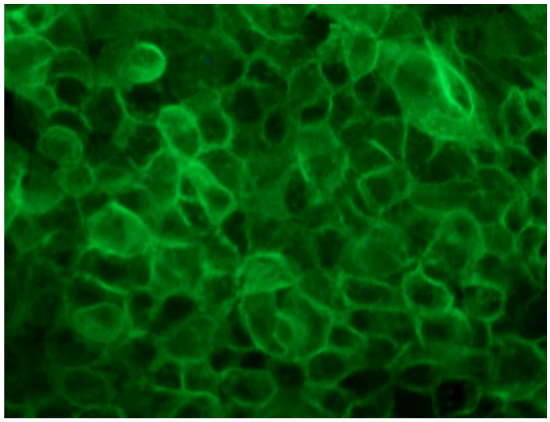
Cells: A431
Lane 1: Mouse IgG2b (isotype control) (M077-3) (5 µL)
Lane 2: Anti-EGF-R (Human) mAb (MI-12-1) (5 µL)
Lane 3: Anti-EGF-R (Human) mAb (MI-12-1) (2 µL)

Immunoblotted with Anti- EGF-R (Human) mAb (MI-12-1)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.
4) The glass slide was washed 3 times with PBS.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS.
7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 100 µL of 1:100 Anti-IgG (H+L chain) (Mouse) pAb-FITC (Beckman Coulter; code no. IM0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A431)



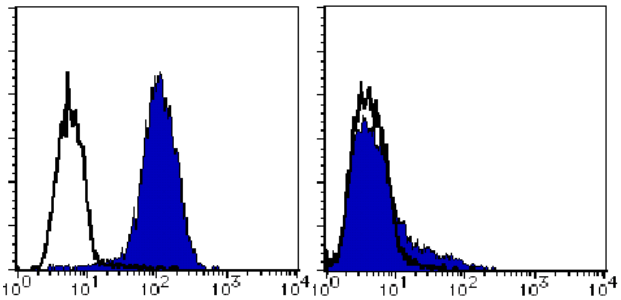
Immunocytochemical detection of human EGF-R on A431 with MI-12-1.

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).

1) Detach the cells from culture dish by using cell dissociation buffer (Thermo Fisher Scientific; code no. 13151-014).
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
3) Add 100 µL of PBS containing 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
4) Wash the cells 3 times with washing buffer.
5) Add 100 µL of 70% ethanol to the cell pellet after tapping. Mix well, then permeablize the cells for 30 minutes at -20°C.
6) Wash the cells 3 times with washing buffer.
7) 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 (20~25°C).
8) Add 40 µL of the anti-EGF-R (Human) mAb (6F1) at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
9) 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.
10) Add 30 µL of 1:100 Anti-IgG (H+L chain) (Mouse) pAb-FITC (Beckman Coulter: code no. IM0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
11) 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.
12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; A431)



Flow cytometric analysis of human EGF-R expression on permeabilized A431 (left) or non-permeabilized A431 (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of MI-12-1 to the cells.*

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