

Anti-HB-EGF (Human) mAb

CODE No.	D308-3
CLONALITY	Monoclonal
CLONE	3H4
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Human HB-EGF, extracellular domain (recombinant)
FORMURATION	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.

APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	0.1 μ g/100 μ L of cell extract from 6×10^5 cells
<u>Immunocytochemistry</u>	1 μ g/mL
<u>Flow cytometry</u>	5 μ g/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant	Transfectant	Not tested	Not tested
Reactivity	+	-		

Entrez Gene ID 1839 (Human)

REFERENCES
1) Hamaoka, M., *et al.*, *J. Biochem.* **148**, 55-69 (2010)
2) Mekada, E. and Iwamoto, R., *UCSD Nature Molecule Pages* doi:10.1038/mp.a002932.01

For more information, please visit our web site <http://ruo.mbl.co.jp/>

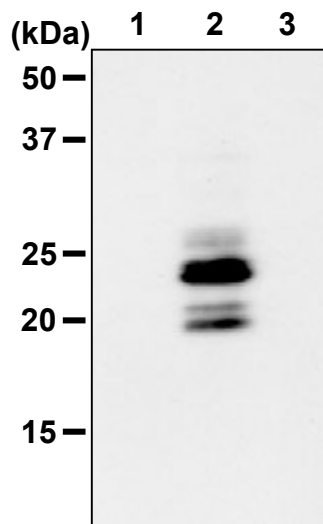
RELATED PRODUCTS

D308-3 Ant-HB-EGF (Human) mAb (3H4)
M220-3 Ant-HB-EGF (Human) mAb (2-108)
MI-12-1 Anti-EGF-R (Human) mAb (6F1)
5346 Ab-Mach Human AREG Assembly Kit
M075-3 Mouse IgG1 (isotype control) (2E12)

SDS-PAGE & Western blotting

- 1) Culture 3×10^6 cells on a 10-cm dish, then incubate in a CO₂ incubator for one night.
- 2) Prepare cell lysate with 300 μ L of ice-cold RIPA buffer [50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 0.5% deoxycholate] or OG-lysis buffer [60 mM octyl-b-D-glucopyranosid, 150 mM NaCl, 20 mM HEPES-NaOH (pH 7.2)] containing appropriate protease inhibitors, then incubate for 30 min. at 4°C.
- 3) Centrifuge the tube at 12,000 x g for 20 min. at 4°C and transfer 150 μ L of the supernatant to another tube.
- 4) Add 75 μ L Laemmli's sample buffer (non-reduced condition) and boil the samples for 5 min. and centrifuge. Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 5) Blot the protein to Immobilon-P (Millipore) at 1.2 mA/cm² for 1.5 hr. in a semi-dry transfer system (Transfer Buffer: 100 mM Tris, 190 mM glycine, 5% MeOH). See the manufacturer's manual for precise transfer procedure.
- 6) Wash the membrane with 10 mL of TBS-T [0.05% Tween-20 in TBS] (3 min. x 3 times).
- 7) To reduce nonspecific binding, soak the membrane in 10 mL of blocking buffer (1% skimmed milk in TBS-T) overnight at 4°C.
- 8) Wash the membrane with 10 mL of TBS-T (3 min. x 2 times).
- 9) Incubate the membrane with 1 mL of primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 10) Wash the membrane with 10 mL of TBS-T (5 min. x 5 times).
- 11) Incubate the membrane with 1 mL of 1:3,000 anti-IgG (Mouse)-HRP (CHEMICON; code no. AP192P) diluted with blocking buffer for 1 hr. at room temperature.
- 12) Wash the membrane with TBS-T (5 min. x 5 times).
- 13) Wipe excess buffer on the membrane, and then incubate it with 1 mL of chemiluminescence reagent for 1 min. 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 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blot analysis of HB-EGF in transfectant

Lane 1: Parental cell (Vero)
Lane 2: Human HB-EGF/Vero
Lane 3: Mouse HB-EGF/Vero

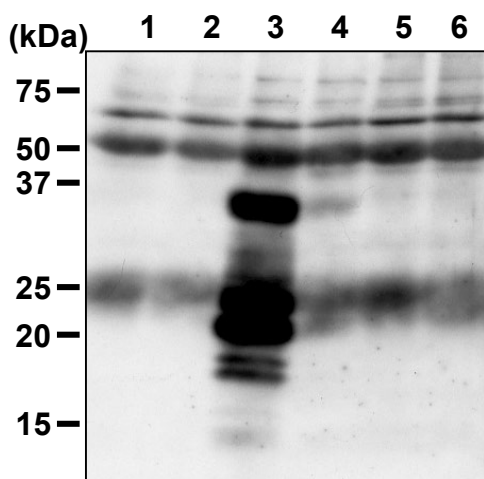
Immunoblotted with D308-3

Samples were kindly provided by Drs. Ryo Iwamoto and Eisuke Mekada.
(Department of Cell Biology, Research Institute for Microbial Diseases,
Osaka University).

Immunoprecipitation

- 1) Culture 3×10^6 cells on a 10-cm dish, then incubate in a CO₂ incubator for one night.
- 2) Wash cells 2 times with 5 mL of PBS (+).
- 3) Incubate cells with 4 mL of 0.2 mg/mL Sulfo-NHS-LC-biotin in ice-cold biotinylation buffer [10 mM HEPES-NaOH (pH 8.0), 150 mM NaCl, 0.2 mM CaCl₂, 0.2 mM MgCl₂] for 30 min. at 4°C.
- 4) Wash cells 2 times with 4 mL of TBS (+) [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2 mM CaCl₂, 0.2 mM MgCl₂].
- 5) Wash cells 2 times with 4 mL of 0.1% BSA/PBS (+).
- 6) Prepare cell lysate with 0.5 mL of ice-cold RIPA buffer [50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 0.5% deoxycholate] or OG-lysis buffer [60 mM octyl-b-D-glucopyranosid, 150 mM NaCl, 20 mM HEPES-NaOH (pH 7.2)] containing appropriate protease inhibitors, then incubate for 30 min. at 4°C.
- 7) Centrifuge the tube at 12,000 x g for 20 min. at 4°C and transfer 100 µL of supernatant to another tube.
- 8) Add primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 4 hr. at 4°C.
- 9) Mix 10 µL of anti-IgG (Mouse)-sepharose beads slurry. Incubate with gentle agitation for 2 hr. at 4°C.
- 10) Wash the beads 4 times with 1 mL of RIPA buffer.
- 11) Resuspend the beads in 40 µL of Laemmli's sample buffer, boil for 10 min. and centrifuge.
- 12) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 13) Blot the protein to Immobilon-P (Millipore) at 1.2 mA/cm² for 1.5 hr. in a semi-dry transfer system (Transfer Buffer: 100 mM Tris, 190 mM glycine, 5% MeOH). See the manufacturer's manual for precise transfer procedure.
- 14) To reduce nonspecific binding, soak the membrane in 10 mL of 3% BSA in TBS-T [0.05% Tween-20 in TBS] for 30 min. at 37°C.
- 15) Wash the membrane with 20 mL of TBS-T (3 min. x 2 times).
- 16) Incubate the membrane with 4 mL of 1:4,000 streptavidin-HRP (ZYMED; code no. 43-8323) diluted with 1% BSA in TBS-T for 1 hr. at room temperature.
- 17) Wash the membrane with 10 mL of TBS-T (5 min. x 5 times).
- 18) Wipe excess buffer on the membrane, and then incubate it with 2 mL of chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 19) Expose to an X-ray film in a dark room for 20 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Transfectant)



Immunoprecipitation of HB-EGF from transfectant

Cell sample

Lane 1 and 2: Parental cell (Vero)

Lane 3 and 4: Human HB-EGF/Vero

Lane 5 and 6: Mouse HB-EGF/Vero

IP antibody amount

Lane 1, 3 and 5: IP with D308-3, 1 µg/mL

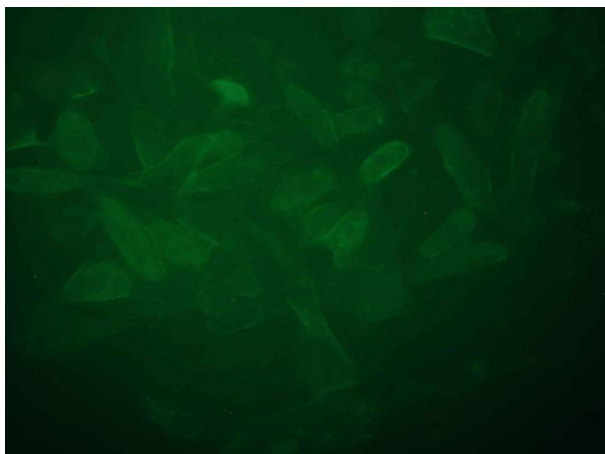
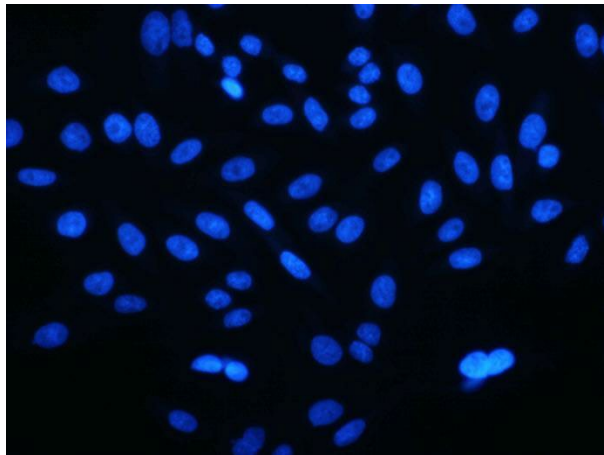
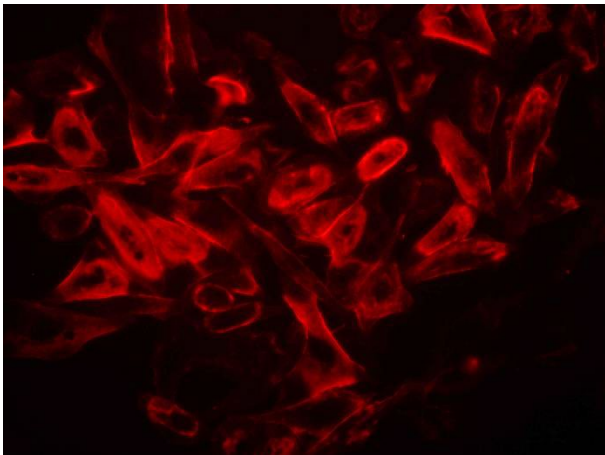
Lane 2, 4 and 6: IP with D308-3, 0.1 µg/mL

Samples were kindly provided by Drs. Ryo Iwamoto and Eisuke Mekada. (Department of Cell Biology, Research Institute for Microbial Diseases, Osaka University).

Immunocytochemistry

- 1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO₂ incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 min., Take care not to touch the cells. Repeat another wash once more.
- 5) Add 200 µL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 min. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 6) Wash the slides 2 times in PBS for 5 min. each.
- 7) Add 200 µL of 1:500 anti-IgG (Mouse)-Alexa Fluor® 594 (Invitrogen; code no. A11020) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 8) Wash the slides 2 times in PBS for 5 min. each.
- 9) Add 200 µL of 1 µg/mL Anti-His-tag mAb-Alexa Fluor® 488 (MBL; code no. D291-A48) diluted with 0.1% Triton X-100 in PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 10) Wash the slides 2 times in PBS for 5 min. each.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Counter stain with DAPI for 5 min. at room temperature.
- 13) Wash the slides 2 times in PBS for 5 min. each.
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Transfectant)



Immunocytochemical detection of Myc-His-tagged human HB-EGF in CHO

Red: D308-3

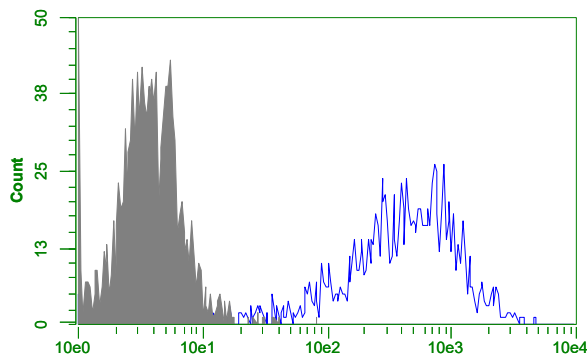
Green: Anti-His-tag mAb-Alexa Fluor® 488
(MBL; code no. D291-A48)

Blue: DAPI

Flow cytometric analysis for adherent cells

- 1) Detach the cells from culture dish.
- 2) Wash the cells (3×10^5 cells/sample) 1 time with 1 mL of washing buffer (2% fetal calf serum (FCS)/PBS).
- 3) Add 10 μ L of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
- 4) Add 30 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 20 min. at 4°C.
- 5) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
- 6) Add PE-conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
- 7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)



Flow cytometric detection of Myc-His-tagged human HB-EGF in 293T

Open: D308-3

Closed: Isotype control (MBL; code no. M075-3)