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

Anti-HB-EGF (Human) mAb

CODE No. D308-3

CLONALITY Monoclonal
CLONE 3H4
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 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

Western blotting	1 µg/mL for chemiluminescence detection system
Immunoprecipitation	0.1 µg/100 µL of cell extract from 6 x 10^5 cells
Immunocytochemistry	1 µg/mL
Flow cytometry	5 µg/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

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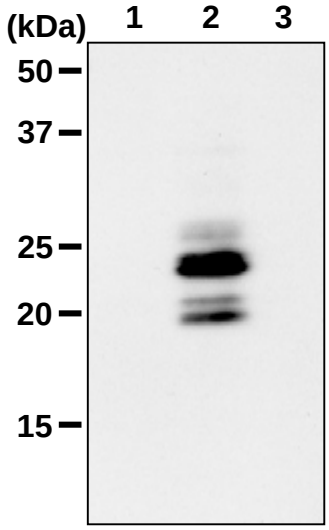
RELATED PRODUCTS

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 $3x10^6$ cells on a 10-cm dish, then incubate in a CO_2 incubator for one night.
2) Prepare cell lysate with 300 μL 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 μL of the supernatant to another tube.
4) Add 75 μL Laemmli's sample buffer (non-reduced condition) and boil the samples for 5 min. and centrifuge. Load 20 μL 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/cm^2 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

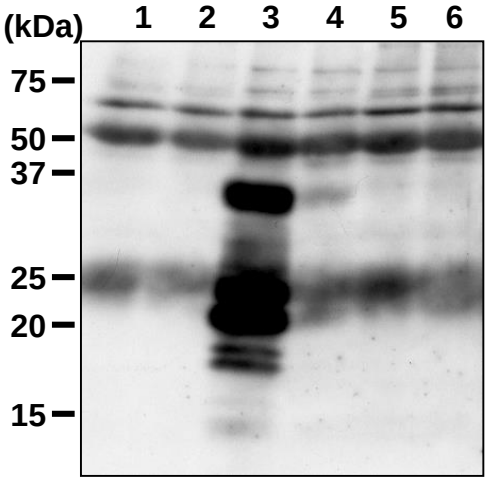
Immunoblotted with D308-3

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 $3x10^6$ cells on a 10-cm dish, then incubate in a CO_2 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 $CaCl_2$, 0.2 mM $MgCl_2$] 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 $CaCl_2$, 0.2 mM $MgCl_2$].
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/cm^2 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

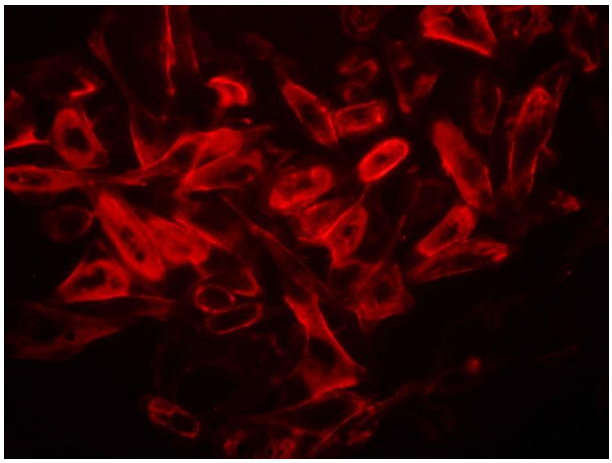
Cell sample
Lane 1 and 2: Parental cell (Vero)
Lane 3 and 4: Human HB-EGF/Vero
Lane 5 and 6: Mouse HB-EGF/Vero
IP antibody amount
Lane 1, 3 and 5: IP with D308-3, 1 μg/mL
Lane 2, 4 and 6: IP with D308-3, 0.1 μg/mL

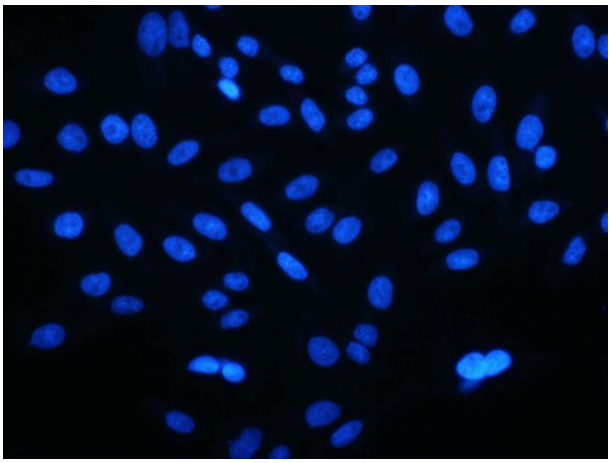
Samples were kindly provided by Drs. Ryo Iwamoto and Eisuke Mekada. (Department of Cell Biology, Research Institute for Microbial Diseases, Osaka University).

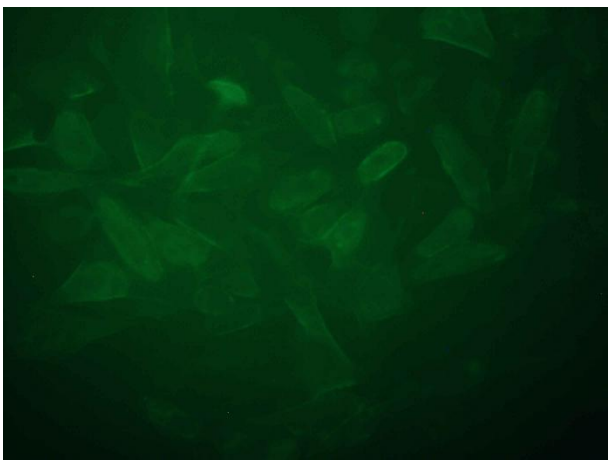
Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 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)





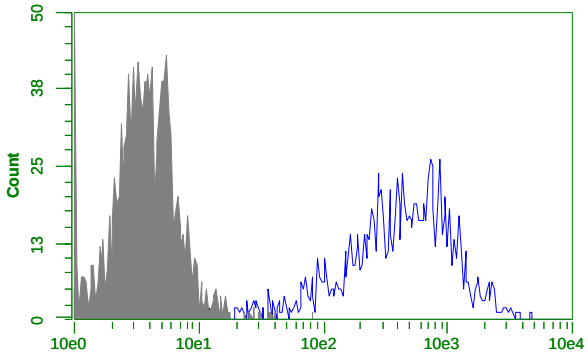


Immunocytochemical detection of
Myc-His-tagged human HB-EGF in CHO
Red: D308-3
Green: Anti-His-tag mAb-Alexa Fluor® 488
(MBL; code no. D291-A48)
Blue: DAPI

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells (3 x 10^5 cells/sample) 1 time with 1 mL of washing buffer (2% fetal calf serum (FCS)/PBS).
3) Add 10 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 10 min. at room temperature.
4) Add 30 µL 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 µL 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)