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

# Anti-HB-EGF mAb

**CODE No.** M220-3

**CLONALITY** Monoclonal
**CLONE** 2-108
**ISOTYPE** Mouse IgG1 κ
**QUANTITY** 100 µL, 1 mg/mL

**SOURCE** Purified IgG from hybridoma supernatant
**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
Immunohistochemistry 1 µg/mL
Heat treatment for paraffin embedded section: Autoclave; 125°C for 5 min. in 10 mM citrate buffer (pH 6.0)

## APPLICATIONS-REPORTED

Immunoprecipitation Reference 1)
Immunocytochemistry Reference 1)
Cell-based ELISA Reference 1)

## SPECIES CROSS REACTIVITY on WB

| Species | Human | Mouse* | Rat | Hamster |
|---|---|---|---|---|
| Cell | Transfectant | Transfectant | Not tested | Not tested |
| Reactivity | + | - | | |

*It is reported in the reference number 1).

**Entrez Gene ID** 1839 (Human)

**REFERENCE**

1) Iwamoto, R., *et al.*, *Monoclon. Antib. Immunodiagn. Immunother.* 35, 73-82 (2016) [WB, IP, IC, IHC, ELISA]

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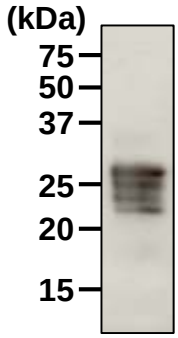
## RELATED PRODUCTS

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

## SDS-PAGE & Western blotting

1) Wash 5 x $10^6$ cells 3 times with PBS and suspend them in 500 μL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the sample for 10 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/$cm^2$ for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in a plastic wrap.
10) Expose to an X-ray film in a dark room for 15 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; 293T transfectant)



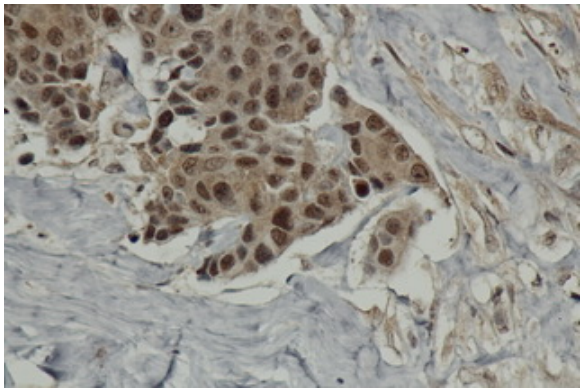

***Western blot analysis of human HB-EGF***
Sample: 293T transfectant

Immunoblotted with Anti-HB-EGF mAb (M220-3)

## Immunohistochemical staining for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 5 min. each.
2) Wash the slides 2 times in 100% Ethanol, 1 time in 95% Ethanol and 1 time in 70% Ethanol for 5 min. each.
3) Wash the slides with PBS 3 times for 5 min. each.
4) Remove the slides from PBS and perfrom heat treatment for antigen retrieval.
   Heat-treatment: 10 mM Citrate buffer (pH 6.0) for 5 min. at 125°C using an autoclave.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Remove the slides from the Citrate buffer and block endogenous peroxidase with 3% $H_2O_2$ in PBS for 10 min.
7) Wash the slides with PBS 3 times for 5 min. each.
8) Remove the slides from PBS, wipe gently around each section and cover tissues with 10% goat serum for 30 min. at room temperature (20~25°C) to block non-specific staining.
9) Wash the slides with PBS 3 times for 5 min. each.
10) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% BSA/PBS as suggested in the **APPLICATIONS** overnight at 4°C. (The concentration of antibody will depend on the conditions.)
11) Wash the slides 3 times in TBS-T [0.05% Tween-20 in TBS] for 5 min. each.
12) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 30 min. at room temperature.
13) Wash the slides 3 times in TBS-T for 5 min. each, and then, wash the slides with water.
14) Visualize by reacting for 2 min. with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Wash the slides in water for 5 min.
16) Counter stain in hematoxylin for 1 min., wash the slides with running tap water.
17) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Human breast cancer tissue)



### *Immunohistochemical detection of HB-EGF in human breast cancer tissue*

Brown: Anti-HB-EGF mAb (M220-3)
Blue: Hematoxylin

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