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

Anti-E-tag pAb

CODE No.	PM070
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 µL
SOURCE	Purified Ig from rabbit serum
IMMUNOGEN	KLH conjugated synthetic peptide, GAPVPYPDPLEPR (E-tag)
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	1:1,000
Immunoprecipitation	2 µL/sample

For more information, please visit our website at **https://ruo.mbl.co.jp/.**

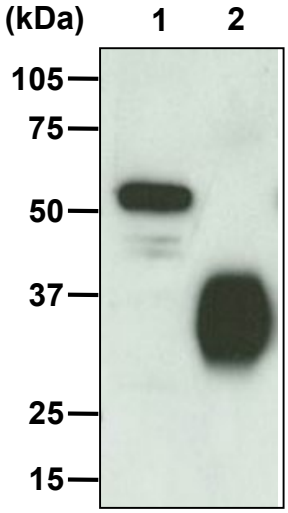
MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
A JSR Life Sciences Company

URL https://ruo.mbl.co.jp e-mail support@mbl.co.jp

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 5 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% 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 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
5) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 of anti-IgG (Rabbit)-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) Wipe excess buffer on the membrane, and 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 plastic wrap.
11) 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.

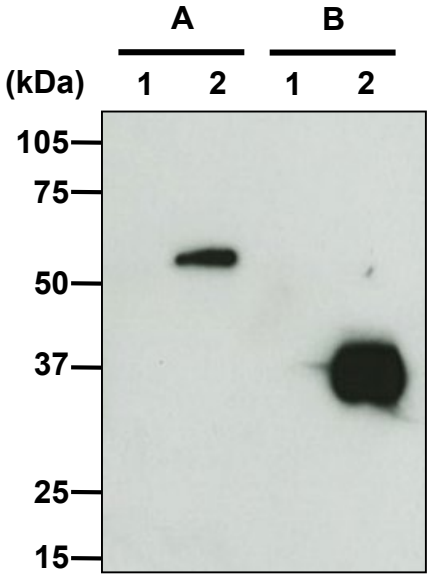


Western blot analysis of E-tagged protein
Lane 1: E-tagged protein X/CHO (Cell lysate)
Lane 2: E-tagged protein Y/CHO (Cell culture supernatant)

Immunoblotted with Anti-E-tag pAb (PM070)

Immunoprecipitation

1) Wash 5 x 10^6 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Extraction buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 20 sec.).
2) Incubate it on ice for 5min.
3) Centrifuge the tube at 12,000 x g for 10 min. at 4°C and transfer the supernatant to another tube.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
5) Wash the beads 3 times with 1 mL of IP buffer.
6) Add 300 µL of cell lysate, then incubate with gentle agitation for 1 hr. at room temperature.
7) Wash the beads 5 times with 1 mL of Extraction buffer.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
9) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
10) 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.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
13) Incubate the membrane with 1:1,000 of anti-E-tag pAb (MBL; code no. PM070) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Incubate the membrane with the 1:1,000 of Rabbit TrueBlot® anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
18) 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 1 min. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of E-tagged protein

A: E-tagged protein X/CHO (Cell lysate)
B: E-tagged protein Y/CHO (Culture supernatant)

1: Normal rabbit IgG (PM035)
2: Anti-E-tag pAb (PM070)

Immunoblotted with Anti-E-tag pAb (PM070)