

**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, GAPVPYDPLEPR (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

<u>Western blotting</u>	1:1,000 for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 µL/sample

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



RELATED PRODUCTS

Antibodies

PM070	Anti-E-tag (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag (polyclonal)
M192-3	Anti-Myc-tag (My3) (200 µL)
M192-3S	Anti-Myc-tag (My3) (50 µL)
M047-3	Anti-Myc-tag (PL14)
M047-6	Anti-Myc-tag-Biotin (PL14)
M047-7	Anti-Myc-tag-HRP-DirecT (PL14)
M047-8	Anti-Myc-tag-Agarose (PL14)
M047-A48	Anti-Myc-tag-Alexa Fluor® 488 (PL14)
M047-A59	Anti-Myc-tag-Alexa Fluor® 594 (PL14)
M047-A64	Anti-Myc-tag-Alexa Fluor® 647 (PL14)
562	Anti-Myc-tag (polyclonal) (0.1 mL)
562-5	Anti-Myc-tag (polyclonal) (0.5 mL)
M180-3	Anti-HA-tag (TANA2) (200 µL)
M180-3S	Anti-HA-tag (TANA2) (50 µL)
M180-7	Anti-HA-tag-HRP-DirecT (TANA2)
M180-A48	Anti-HA-tag-Alexa Fluor® 488 (TANA2)
M180-A59	Anti-HA-tag-Alexa Fluor® 594 (TANA2)
M180-A64	Anti-HA-tag-Alexa Fluor® 647 (TANA2)
561	Anti-HA-tag (polyclonal) (0.1 mL)
561-5	Anti-HA-tag (polyclonal) (0.5 mL)
561-7	Anti-HA-tag-HRP-DirecT (polyclonal)
561-8	Anti-HA-tag-Agarose (polyclonal)
M132-3	Anti-HA-tag (5D8)
M185-3L	Anti-DDDDK-tag (FLA-1) (1 mL)
M185-3LL	Anti-DDDDK-tag (FLA-1) (5 mL)
M185-3S	Anti-DDDDK-tag (FLA-1) (50 µL)
M185-A48	Anti-DDDDK-tag-Alexa Fluor® 488 (FLA-1)
M185-A59	Anti-DDDDK-tag-Alexa Fluor® 594 (FLA-1)
M185-A64	Anti-DDDDK-tag-Alexa Fluor® 647 (FLA-1)
PM020	Anti-DDDDK-tag (polyclonal)
PM020-7	Anti-DDDDK-tag-HRP-DirecT (polyclonal)
PM020-8	Anti-DDDDK-tag-Agarose (polyclonal)
D291-3	Anti-His-tag (OGHis) (200 µL)
D291-3S	Anti-His-tag (OGHis) (50 µL)
D291-6	Anti-His-tag-Biotin (OGHis)
D291-7	Anti-His-tag-HRP-DirecT (OGHis)
D291-8	Anti-His-tag-Agarose (OGHis)
D291-A48	Anti-His-tag-Alexa Fluor® 488 (OGHis)
D291-A59	Anti-His-tag-Alexa Fluor® 594 (OGHis)
D291-A64	Anti-His-tag-Alexa Fluor® 647 (OGHis)
M089-3	Anti-His-tag (6C4)
M136-3	Anti-His-tag (2D8)
PM032	Anti-His-tag (polyclonal)
PM032-8	Anti-His-tag-Agarose (polyclonal)
598	Anti-GFP (polyclonal)
598-7	Anti-GFP-HRP-DirecT (polyclonal)
M048-3	Anti-GFP (1E4)
D153-3	Anti-GFP (RQ2)
D153-A48	Anti-GFP-Alexa Fluor® 488 (RQ2)
D153-A59	Anti-GFP-Alexa Fluor® 594 (RQ2)
D153-A64	Anti-GFP-Alexa Fluor® 647 (RQ2)
D153-8	Anti-GFP-Agarose (RQ2)
PM005	Anti-RFP (polyclonal)
PM005-7	Anti-RFP-HRP-DirecT (polyclonal)
M155-3	Anti-RFP (8D6)
M165-3	Anti-RFP (3G5)

M165-8	Anti-RFP-agarose (3G5)
M167-3	Anti-V5-tag (1H6)
PM003	Anti-V5-tag (polyclonal)
PM003-7	Anti-V5-tag-HRP-DirecT (polyclonal)
PM003-8	Anti-V5-tag-Agarose (polyclonal)

Smart-IP series

3190	Magnetic Rack
M198-9	Anti-E-tag-Magnetic beads (21D11)
M185-9	Anti-DDDDK-tag-Magnetic beads (FLA-1)
D291-9	Anti-His-tag-Magnetic beads (OGHis)
D153-9	Anti-GFP-Magnetic beads (RQ2)
M165-9	Anti-RFP-Magnetic beads (3G5)
M132-9	Anti-HA-tag-Magnetic beads (5D8)
M180-9	Anti-HA-tag-Magnetic beads (TANA2)
M047-9	Anti-Myc-tag-Magnetic beads (PL14)
M167-9	Anti-V5-tag-Magnetic beads (1H6)
D058-9	Anti-Multi Ubiquitin-Magnetic beads (FK2)
M075-9	Mouse IgG1 (isotype control)-Magnetic beads
M076-9	Mouse IgG2a (isotype control)-Magnetic beads
M077-9	Mouse IgG2b (isotype control)-Magnetic beads
M081-9	Rat IgG2a (isotype control)-Magnetic beads
M198-10	Anti-E-tag-Magnetic Agarose (21D11)
M185-10	Anti-DDDDK-tag-Magnetic Agarose (FLA-1)
D291-10	Anti-His-tag-Magnetic Agarose (OGHis)
D153-10	Anti-GFP-Magnetic Agarose (RQ2)
M165-10	Anti-RFP-Magnetic Agarose (3G5)
M132-10	Anti-HA-tag-Magnetic Agarose (5D8)
M180-10	Anti-HA-tag-Magnetic Agarose (TANA2)
M047-10	Anti-Myc-tag-Magnetic Agarose (PL14)
M167-10	Anti-V5-tag-Magnetic Agarose (1H6)

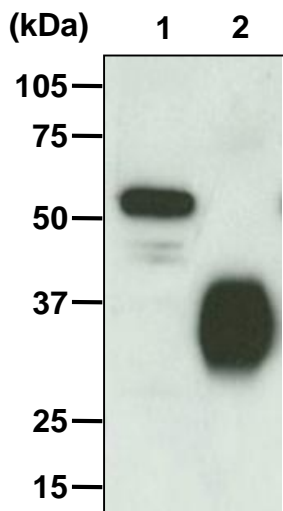
Protein Purification Kit

3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3307	c-Myc-tagged Protein MILD PURIFICATION GEL (5 mL gel, 5 mg peptide)
3300-205	c-Myc tag peptide (5 mg)
3310	His-tagged Protein PURIFICATION KIT
3310-205	His-tag peptide (10mg)
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 10 mg peptide)
3312	His-tagged Protein PURIFICATION GEL (5 mL gel, 50 mg peptide)
3315	V5-tagged Protein PURIFICATION KIT
3320	HA-tagged Protein PURIFICATION KIT
3325	DDDDK-tagged Protein PURIFICATION KIT
3325-205	DDDDK-tag peptide (5 mg)
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3327	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel, 25 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3329	DDDDK-tagged Protein PURIFICATION GEL (25 mL gel)

Other related antibodies and kits are also available.
Please visit our website at <http://ruo.mbl.co.jp/>

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend 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 μ L 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/cm² 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

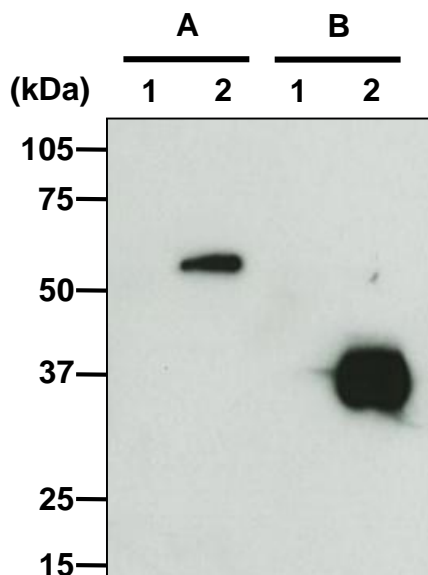
Lane 1: E-tagged protein X/CHO (Cell lysate)

Lane 2: E-tagged protein Y/CHO (Cell culture supernatant)

Immunoblotted with Anti-E-tag pAb (PM070)

Immunoprecipitation

- 1) Wash 5×10^6 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 5 min.
- 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 μ L of 50% protein A agarose beads slurry resuspended in 400 μ L 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 μ L 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 μ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 9) Load 10 μ L 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/cm² 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)