

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



Anti-Calmodulin Binding Protein-tag pAb

| | |
|--------------------|---|
| CODE No. | PM071 |
| CLONALITY | Polyclonal |
| ISOTYPE | Rabbit Ig, affinity purified |
| QUANTITY | 100 µL |
| SOURCE | Purified Ig from rabbit serum |
| IMMUNOGEN | KLH conjugated synthetic peptide, KRRWKKNFIAVSAANRFKKISSSGAL (CBP-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> | 2-5 µL/sample |

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



RELATED PRODUCTS

Antibodies

| | |
|----------|--|
| PM071 | Anti-Calmodulin Binding Protein-tag (polyclonal) |
| PM070 | Anti-E-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) |

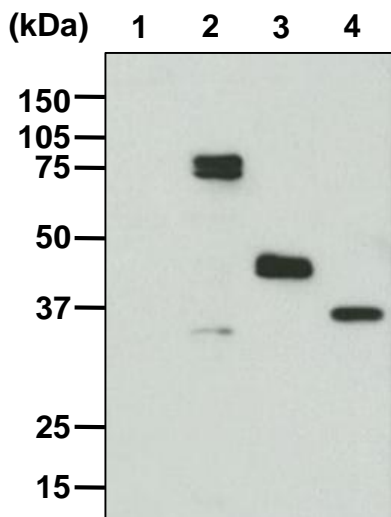
Protein Purification Kit

| | |
|----------|---|
| 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 10 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 Calmodulin Binding Protein

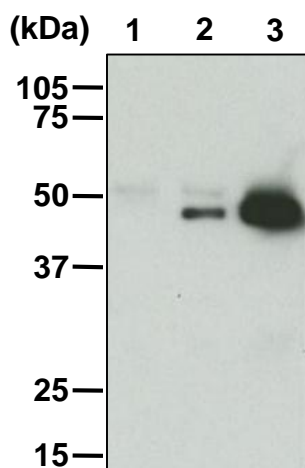
(CBP)-tagged protein

- Lane 1: Parental cell (293T)
- Lane 2: N-terminal CBP-tagged protein X/293T
- Lane 3: C-terminal CBP-tagged protein Y/293T
- Lane 4: C-terminal CBP-tagged protein Z/293T

Immunoblotted with Anti-Calmodulin Binding Protein-tag pAb (PM071)

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 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 μ L of 50% protein A agarose beads slurry resuspended in 300 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 400 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-Calmodulin Binding Protein-tag pAb (MBL; code no. PM071) 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 Calmodulin Binding Protein (CBP)-tagged protein

Sample: C-terminal CBP-tagged protein Y/293T

Lane 1: Normal rabbit IgG (PM035)

Lane 2: Anti-Calmodulin Binding Protein-tag pAb (PM071, 2 μ L/sample)

Lane 3: Anti-Calmodulin Binding Protein-tag pAb (PM071, 5 μ L/sample)

Immunoblotted with PM071