Smart-IP Series

Anti-DDDDK-tag mAb-Magnetic Agarose

CODE No. M185-10

CLONALITY Monoclonal
CLONE FLA-1
ISOTYPE Mouse IgG2a κ
QUANTITY 100 tests (Gel: 1 mL)

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH conjugated synthetic peptide, DYKDDDDK (DDDDK-tag)
REACTIVITY This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged proteins.
FORMULATION 2 mg of antibody is covalently coupled to 1 mL of magnetic agarose gel and provided as
2 mL gel slurry suspended in PBS/0.1% ProClin 150
STORAGE This gel slurry is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED
  Immunoprecipitation 10 μL of gel/400 μL of cell extract from 1 x 10^6 cells

REFERENCES

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

**Smart-IP series**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>3190</td>
<td>Magnetic Rack</td>
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<tr>
<td>M047-10</td>
<td>Anti-Myc-tag mAb-Magnetic Agarose (PL14)</td>
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<tr>
<td>M185-10</td>
<td>Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)</td>
</tr>
<tr>
<td>D291-10</td>
<td>Anti-His-tag mAb-Magnetic Agarose (OGHis)</td>
</tr>
<tr>
<td>D135-10</td>
<td>Anti-GFP mAb-Magnetic Agarose (RQ2)</td>
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<td>M165-10</td>
<td>Anti-RFP mAb-Magnetic Agarose (3G5)</td>
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<td>Anti-HA-tag mAb-Magnetic Agarose (5D8)</td>
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<td>M180-10</td>
<td>Anti-HA-tag mAb-Magnetic Agarose (TANA2)</td>
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<td>M167-10</td>
<td>Anti-V5-tag mAb-Magnetic Agarose (1H6)</td>
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<td>M198-9</td>
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<td>D058-11</td>
<td>Anti-Multi Ubiquitin mAb-Magnetic Beads (FK2)</td>
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**Antibodies**

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<td>Anti-Myc-tag mAb-Agarose (PL14)</td>
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<td>M185-3S</td>
<td>Anti-DDDDK-tag mAb (FLA-1) (50 μL)</td>
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<td>Anti-DDDDK-tag mAb-Biotin (FLA-1)</td>
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<td>M214-3</td>
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<td>M214-7</td>
<td>Anti-mini-AID-tag mAb-HRP-DirecT (1E4)</td>
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**Protein Purification Kits**

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<tr>
<td>3305</td>
<td>c-Myc-tagged Protein MILD PURIFICATION KIT</td>
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<tr>
<td>3340</td>
<td>c-Myc-tagged Protein Magnetic PURIFICATION KIT</td>
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<tr>
<td>3306</td>
<td>c-Myc-tagged Protein MILD PURIFICATION GEL</td>
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<tr>
<td>3300-205</td>
<td>with Elution Peptide (1 mL gel, 1 mg peptide)</td>
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<td>3310</td>
<td>His-tagged Protein PURIFICATION KIT</td>
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<td>3310-205</td>
<td>His-tag peptide (10 mg)</td>
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<td>HA-tagged Protein Purification Gel (1 mL)</td>
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<td>DDDDK-tagged Protein PURIFICATION KIT</td>
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<td>with Elution Peptide (1 mL gel, 5 mg peptide)</td>
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<tr>
<td>3328</td>
<td>DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)</td>
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</table>

Other related antibodies and kits are also available. Please visit our website at [http://ruo.mbl.co.jp/](http://ruo.mbl.co.jp/)
**Immunoprecipitation**

1) Wash 1 x 10^6 cells 3 times with PBS and suspends them in 400 μL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40], then sonicate briefly (up to 10 sec.).

2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.

3) Add magnetic beads as suggested in the APPLICATION into 400 μL of the cell lysate. Mix well and incubate with gentle agitation for 30 min. at 4°C.

4) Place the tube on the magnetic rack (MBL; code no. 3190) for a few seconds.

5) Remove the supernatant.

6) Wash the beads 4 times with 1 mL of cold Lysis buffer (place the tube on the magnetic rack for a few seconds).

7) Resuspend the magnetic beads in 50 μL of Laemmli’s sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.

8) Load 5 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.

9) 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.

10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.

11) Incubate the membrane with 1:10,000 of Anti-DDDDK-tag mAb-HRP-DirecT (MBL; code no. M185-7) diluted with 1% skimmed milk (in PBS, pH 7.2) PBS for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)

12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).

13) 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 plastic wrap.

14) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual settings. The condition for exposure and development may vary.

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**Immunoprecipitation of DDDDK-tagged protein**

- **Lane 1:** 293T cell lysate
- **Lane 2:** N-terminal DDDDK-tagged protein X (1 μg) in 293T lysate
- **Lane 3:** Internal DDDK-tagged GFP (1 μg) in 293T lysate
- **Lane 4:** C-terminal DDDDK-tagged protein X (1 μg) in 293T lysate

Immunoblotted with Anti-DDDDK-tag mAb-HRP-DirecT (MBL; code no. M185-7)