Anti-IDH1 mAb

**CODE No.** D309-3

**CLONALITY** Monoclonal

**CLONE** RMaB-3

**ISOTYPE** Mouse IgG1 κ

**QUANTITY** 100 μL, 1 mg/mL

**SOURCE** Purified IgG from hybridoma supernatant

**REACTIVITY** This clone reacts with wild type and mutated IDH1.

**FORMURATION** 1 mg/mL in PBS containing 50% glycerol. 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: 1-5 μg/mL for chemiluminescence detection system
- Immunoprecipitation: 5 μg/100 μg lysate
- Immunohistochemistry: 5 μg/mL (paraffin section)
  - Heat treatment for paraffin embedded section: microwave oven, for 10 min. in 10 mM citrate buffer (pH 6.0)

**SPECIES CROSS REACTIVITY on WB**

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>Recombinant protein</td>
<td>Not tested</td>
<td>Not tested</td>
<td>CHO</td>
</tr>
<tr>
<td>Reactivity</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

**Entrez Gene ID** 3417 (Human)

**REFERENCES**

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

D309-3  Anti-IDH1 mAb (RMab-3)
D299-3  Anti-IDH1-R132H (Human) mAb (HMab-1)
D300-3  Anti-IDH1-R132S (Human) mAb (SMab-1)
D331-3  Anti-IDH1-R132G (Human) mAb (GMab-r1)
D311-3  Anti-IDH2 mAb (RMab-22)
D330-3  Anti-IDH2 mAb (KrMab-3)
D328-3  Anti-IDH2-R172K (Human) mAb (KMab-1)

8469  Histostar™ DAB Substrate Solution
SDS-PAGE & Western blotting

1) The recombinant protein is dissolved in Laemmli’s sample buffer at 20 μg/mL.
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/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 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 APPLICATIONS 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 the 1:10,000 of HRP-conjugated anti-mouse IgG (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, 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.
10) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; recombinant human IDH1)
**Immunohistochemistry for formalin fixed paraffin-embedded section**

1) Deparaffinize the sections with Xylene 3 times for 5 min. each.

2) Wash the slides with Ethanol 3 times for 5 min. each.

3) Wash the slides with PBS 3 times for 5 min. each.

4) Heat treatment

   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with plastic wrap, then process the slides for 5 min. each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 min.

5) Remove the slides from the retrieval solution and cover each section with 3% H$_2$O$_2$ in PBS for 10 min. at room temperature to block endogenous peroxidase activity. Wash 2 times in PBS for 5 min. each.

6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (LSAB™ Kit, DAKO; code no. K0690) for 5 min. at room temperature to block non-specific staining. Do not wash.

7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggest in the APPLICATIONS. (The concentration of antibody will depend on the conditions.) Incubate the sections for 1 hr. at room temperature.

8) Wash the slides 2 times in PBS for 5 min. each.

9) Wipe gently around each section and cover tissues with secondary antibody which is attached to LSAB™ Kit (DAKO; code no. K0690). Incubate for 1 hr. at room temperature.

10) Wash the slides 2 times in PBS for 5 min. each.

11) Visualize by reacting for 10 min. with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.

12) Wash the slides in water for 5 min.

13) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min. Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each.

14) Now ready for mounting.

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**Immunohistochemical detection of IDH1**

Human glioblastoma

Immunohistochemical staining with D309-3