

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

Anti-IDH1-R132H (Human) mAb

CODE No.	D299-3
CLONALITY	Monoclonal
CLONE	HMAb-1
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
REACTIVITY	This clone reacts with mutated IDH1-R132H and does not cross-react with wild type IDH1 and other IDH1 mutants.
FORMULATION	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

<u>Western blotting</u>	5-10 μ g/mL
<u>Immunoprecipitation</u>	10 μ g/100 μ g lysate
<u>Immunohistochemistry</u>	5 μ g/mL (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 10 min. in 10 mM citrate buffer (pH 6.0)	
<u>Immunocytochemistry</u>	5 μ g/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Recombinant protein	Not tested	Not tested	CHO
Reactivity	+			-

Entrez Gene ID 3417 (Human)

REFERENCES

- 1) Ishikawa, E., *et al.*, *J. Neurosurg.* **121**, 543-553 (2014) [IHC]
- 2) Takano, S., *et al.*, *J. Neurooncol.* **108**, 361-373 (2012) [IHC]
- 3) Parsons, D. W., *et al.*, *Science* **321**, 1807-1812 (2008)

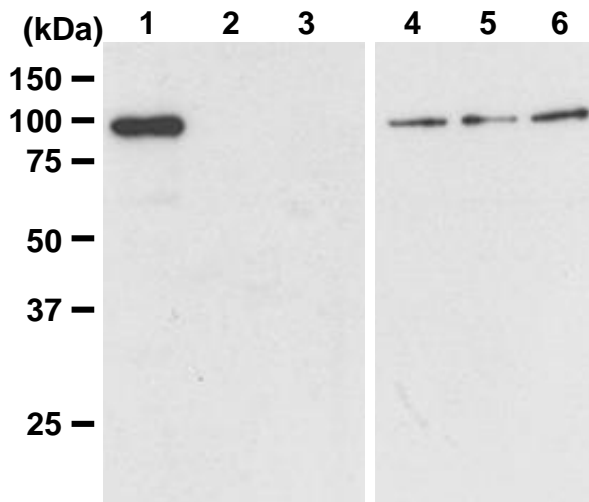
RELATED PRODUCTS

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

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 10 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 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-R132H)



Western blot analysis of IDH1-R132H

Lane 1 and 4: MBP-IDH1-R132H

Lane 2 and 5: MBP-IDH1-R132S

Lane 3 and 6: MBP-IDH1-Wild type

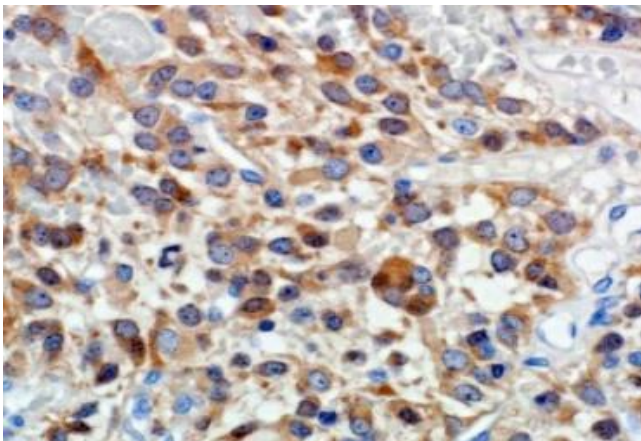
Immunoblot

Lane 1-3: Anti-IDH1-R132H (Human) mAb (D299-3)

Lane 4-6: Anti-MBP mAb (MBL; code no. M091-3)

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% H₂O₂ 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.



Immunohistochemical detection of IDH1

Sample: Human anaplastic oligoastrocytoma
Brown: Anti-IDH1-R132H (Human) mAb
Blue: Hematoxylin