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

Anti-IDH1-R132S

CODE No. D300-3

CLONALITY Monoclonal
CLONE SMab-1
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
REACTIVITY This clone reacts with mutated IDH1-R132S and does not cross-react with wild type IDH1 and other IDH1 mutants.
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 0.5-1 µg/mL for chemiluminescence detection system
Immunoprecipitation 5 µg/100 µg lysate
Immunohistochemistry 1-5 µg/mL (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 10 minutes in 10 mM citrate buffer (pH 6.0)
Immunocytochemistry 1-5 µg/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) Takano, S., *et al.*, *J. Neurooncol.* In press (2012) [IHC]
2) Kaneko, M. K., *et al.*, *Biochem. Biophys. Res. Commun.* **406**, 608-613 (2011) [WB, IC, IHC]

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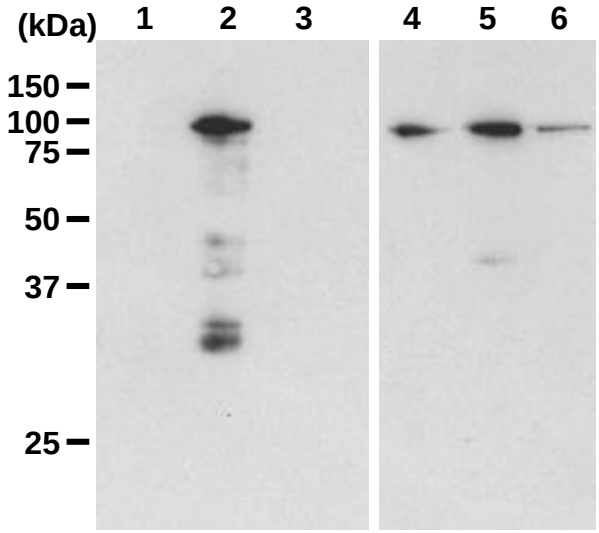
RELATED PRODUCTS

D299-3	anti-IDH1-R132H (HMab-1)
D309-3	anti-IDH1 (RMab-3)
D311-3	anti-IDH2 (RMab-22)
8469	Histostar™ DAB Substrate Solution

SDS-PAGE & Western blotting

1) The recombinant protein is dissolved in Laemmli's sample buffer at 5 μg/mL.
2) Boil the samples for 3 minutes 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/cm^2 for 1 hour 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 hour 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 hour 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 minutes 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 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute. 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 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; recombinant human IDH1-R132S)



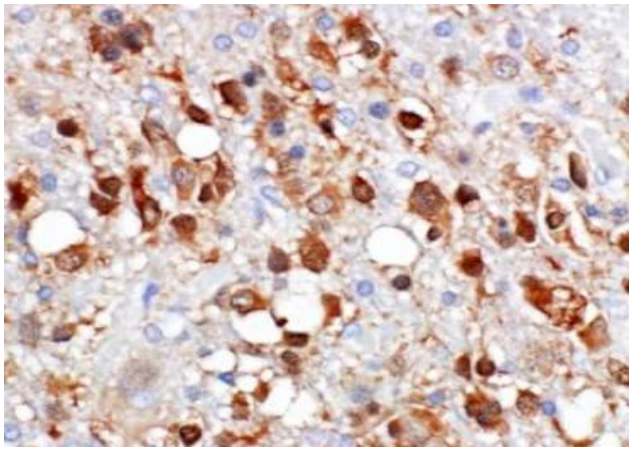
Western blot analysis of IDH1-R132H

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: D300-3
Lane 4-6: anti-MBP (MBL; code no. M091-3)

Immunohistochemistry for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 5 minutes each.
2) Wash the slides with Ethanol 3 times for 5 minutes each.
3) Wash the slides with PBS 3 times for 5 minutes 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 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the retrieval solution and cover each section with 3% H_2O_2 in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 2 times in PBS for 5 minutes 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 minutes 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 hour at room temperature.
8) Wash the slides 2 times in PBS for 5 minutes 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 hour at room temperature.
10) Wash the slides 2 times in PBS for 5 minutes each.
11) Visualize by reacting for 10 minutes 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 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.



Immunohistochemical detection of IDH1
Human anaplastic oligoastrocytoma
Immunohistochemical staining with D300-3