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

Anti-IDH1-R132G (Human) mAb

CODE No. D331-3

CLONALITY Monoclonal
CLONE GMab-r1
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
REACTIVITY This clone reacts with mutated IDH1-R132G and does not cross-react with wild type IDH1 or other IDH1 mutants.
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

Western blotting	1-5 µg/mL for chemiluminescence detection system
Immunocytochemistry	5 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Recombinant protein, transfectant	Not tested	Not tested	Not tested
Reactivity	+			

Entrez Gene ID 3417 (Human)

REFERENCES

1) Takano, S, *et al.*, *Brain Tumor Pathol.* **28**, 115-23 (2011)
2) Kato Y, *et al.*, *Biochem. Biophys. Res. Commun.* **390**, 547-51 (2009)

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

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

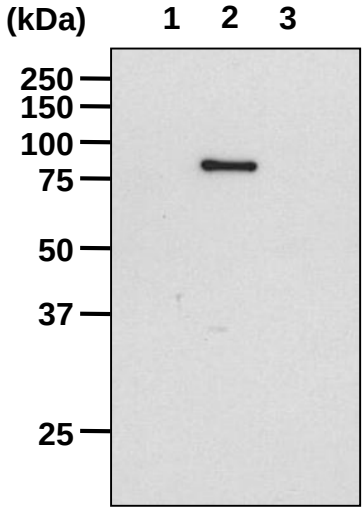
RELATED PRODUCTS

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

SDS-PAGE & Western blotting

1) The recombinant protein is dissolved in Laemmli's sample buffer at 10 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/cm^2 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 anti-IgG (Rat)-HRP (MBL; code no. IM-0825) 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 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; recombinant protein)



Western blot analysis of IDH1-R132G

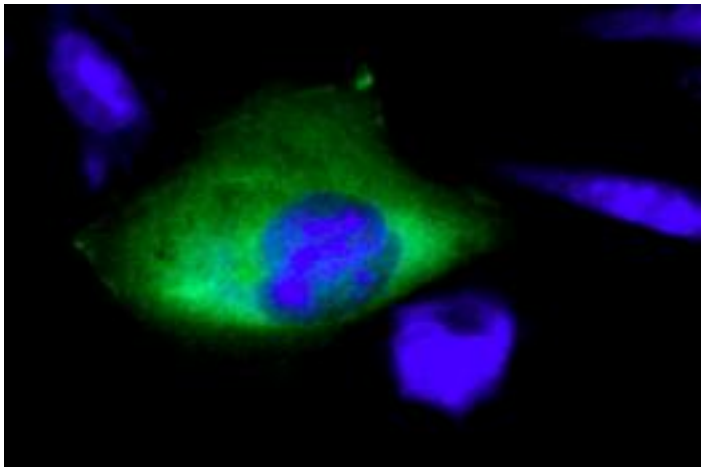
Lane 1: IDH1 (Wild type)
Lane 2: IDH1-R132G
Lane 3: IDH1-R132L

Immunoblotted with Anti-IDH1-R132G mAb (D331-3)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide 1 time in PBS.
4) Fix the cells by immersing the slide in Fixation solution [4% paraformaldehyde (PFA), 0.1 M phosphate buffer (pH 7.4)] for 20 min. at room temperature (20~25°C).
5) Wash the slide 2 times in PBS.
6) Permeabilize the cells with 0.1% Triton-X in PBS for 15 min. at room temperature.
7) Wash the slide 2 times in PBS.
8) Add Blocking buffer (10% normal goat serum in PBS) onto the cells and incubate for 5 min. at room temperature.
9) Tip off the Blocking buffer and add 200 μL of the primary antibody diluted with 0.1% Triton-X in PBS as suggested in the **APPLICATIONS** onto the cells and incubate for overnight at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
10) Wash the slide 1 time with PBS.
11) Wipe excess liquid from the slide but take care not to touch the cells. Add the 1:400 of Alexa Fluor® 488 Donkey Anti-Rat IgG (H+L) (Life Technologies; code no. A-21208) diluted with 0.1% Triton-X in PBS onto the cells and incubate for 1 hr. at room temperature.
12) Wash the slide 1 time with PBS.
13) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
14) Counterstain with 1:200 of TO-PRO®-3 Iodide (642⁄661) (Life Technologies; code no. T3605) diluted with PBS for 1 hr. at room temperature.
15) Wash the slide with PBS for 30 min.
16) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; transfectant)



Immunocytochemical detection of IDH1-R132G in CHO transfectant
Green: IDH1-R132G (D331-3)
Blue: TO-PRO-3