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

Anti-IDH2 mAb

CODE No. D330-3

CLONALITY Monoclonal
CLONE KrMab-3
ISOTYPE Mouse IgG2b κ
QUANTITY 100 μL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
REACTIVITY This clone reacts with wild type and all mutated IDH2 proteins.
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	Jurkat, Raji, U251, HeLa*, Recombinant protein	Not tested	Not tested	CHO
Reactivity	+			+

*very weak reactivity

Entrez Gene ID 3418 (Human)

REFERENCES

1) Kaneko, M. K., *et al.*, *Biochem. Biophys. Res. Commun.* In press (2013) [WB, IC]
2) Parsons, D. W., *et al.*, *Science* **321**, 1807-1812 (2008)

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

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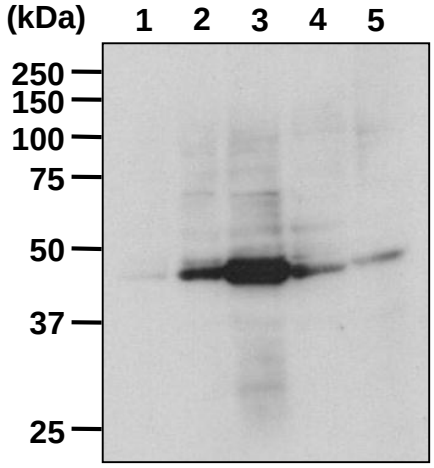
RELATED PRODUCTS

D330-3	Anti-IDH2 mAb (KrMab-3)
D311-3	Anti-IDH2 mAb (RMab-22)
D328-3	Anti-IDH2-R172K (Human) mAb (KMab-1)
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)

SDS-PAGE & Western blotting

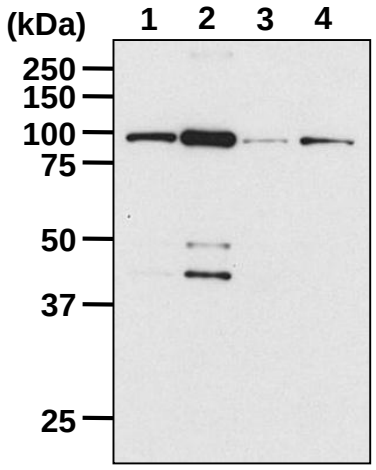
1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
4) 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.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
6) 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.)
7) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
8) Incubate the membrane with the 1:10,000 of anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) 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.
11) 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 controls for Western blotting; Jurkat, Raji, U251, CHO and recombinant protein)



Western blot analysis of IDH2 from cell lines

Lane 1: HeLa
Lane 2: Jurkat
Lane 3: Raji
Lane 4: U251
Lane 5: CHO

Immunoblot with Anti-IDH2 mAb (D330-3)



Western blot analysis of recombinant IDH2 proteins

Loading volume: 100 ng/10 μL

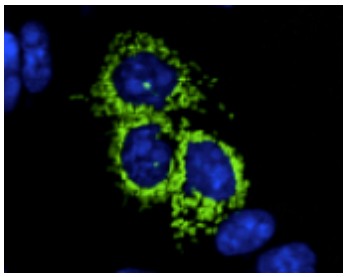
Lane 1: IDH2 (Wild type)
Lane 2: IDH2-R172K
Lane 3: IDH2-R172W
Lane 4: IDH2-R172M

Immunoblot with Anti-IDH2 mAb (D330-3)

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 Goat Anti-Mouse IgG (Life Technologies; code no. A-11029) 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 IDH2 in CHO transfectant
Green: D330-3
Blue: TO-PRO-3