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

Anti-Jmjd1c (Mouse) mAb

CODE No. D356-3

CLONALITY Monoclonal
CLONE 13B
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from mouse ascites fluid
IMMUNOGEN His-tagged Jmjd1c fusion protein corresponding to 245-453 amino acids
FORMULATION 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 µg/mL
Immunohistochemistry 5 µg/mL
Heat treatment for paraffin embedded section: Microwave oven; 2 times for 10 min. each in 10 mM citrate buffer (pH 6.2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Not tested	NIH/3T3, MEF	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 108829 (Mouse)

REFERENCE
1) Kuroki, S., *et al*., *Biol. Reprod*. **89**, 93 (2013)

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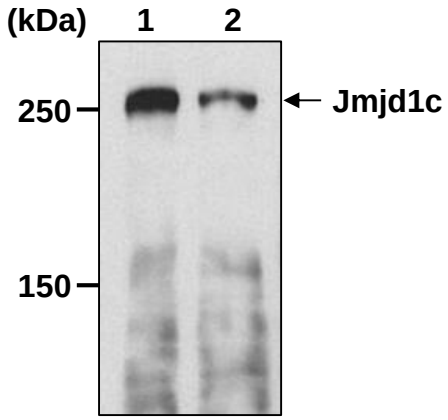
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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) Boil the sample for 2 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 75 min. 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) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 Anti-IgG (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 a plastic wrap.
11) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; NIH/3T3 and MEF)



Western blot analysis of mouse Jmjd1c

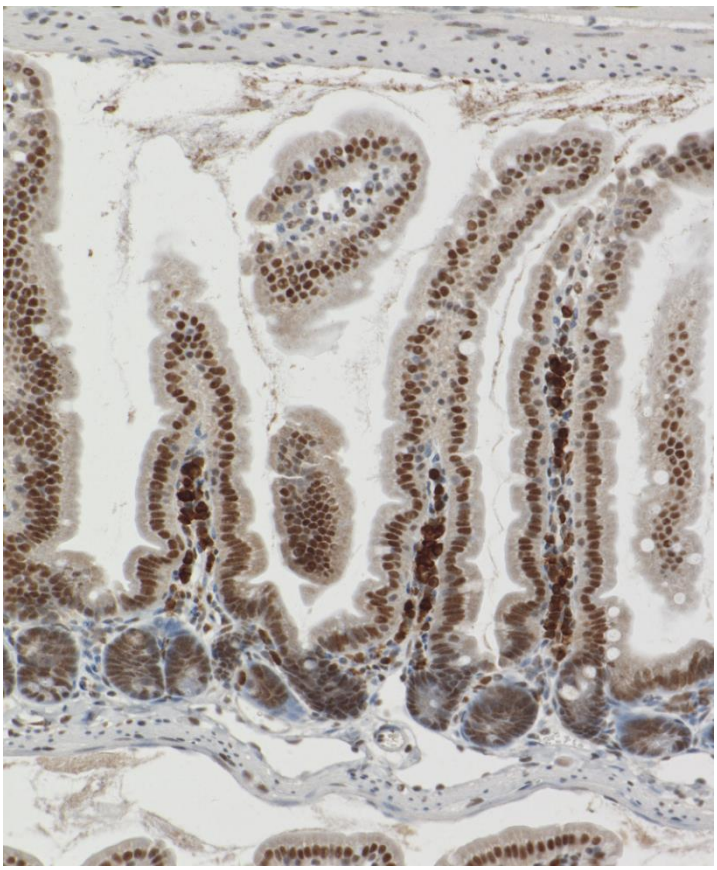
Lane 1: NIH/3T3
Lane 2: MEF

Immunoblotted with Anti-Jmjd1c (Mouse) mAb (D356-3)

Immunohistochemical staining 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) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH 6.2) 2 times for 10 min. using microwave oven.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Remove the slides from the Citrate buffer and block endogenous peroxidase with 3% H_2O_2 in PBS for 10 min.
7) Wash the slides with PBS 2 times for 5 min. each.
8) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 min. at room temperature (20~25°C) to block non-specific staining. Do not wash.
9) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% BSA/PBS as suggested in the **APPLICATIONS** for 1 hr. at 4°C. (The concentration of antibody will depend on the conditions.)
10) Wash the slides 3 times in PBS for 5 min. each.
11) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hr. at room temperature.
12) Wash the slides 3 times in PBS for 5 min. each.
13) Visualize by reacting for 5 min. with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 min.
15) 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.
16) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse intestinal tract)



Immunohistochemical detection of Jmjd1c in mouse intestinal tract
Brown: Anti-Jmjd1c (Mouse) mAb (D356-3)
Blue: Hematoxylin