

Anti-PIWI2 (MILI) mAb

CODE No. D365-3

CLONALITY Monoclonal
CLONE 3G5-E6
ISOTYPE Mouse IgG1 κ
QUANTITY 100 μ L, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN FORMURATION Recombinant protein, corresponding to amino acids 1-200 of mouse PIWI2 (MILI) 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 μ g/mL for chemiluminescence detection system
Immunoprecipitation 2 μ g/sample
Immunohistochemistry 1 μ g/mL

Heat treatment for paraffin embedded section: Microwave oven; 2 times for 10 min. in citrate buffer (pH 6.0),

SPECIES CROSS REACTIVITY on WB

Species	Human*	Mouse	Rat	Hamster
Tissue	Testis	Testis	Not tested	Not tested
Reactivity	-	+		

*Information from the licenser

Entrez Gene ID 57746 (Mouse)

REFERENCES

- 1) Manakov, S. A., *et al.*, *Cell Rep.* **12**, 1234-1243 (2015)
- 2) Aravin, A., *et al.*, *Science* **316**, 744-747 (2007)
- 3) Aravin, A., *et al.*, *Nature* **442**, 203-207 (2006)
- 4) Kuramochi-Miyagawa, S., *et al.*, *Development* **131**, 839-849 (2004)
- 5) Kuramochi-Miyagawa, S., *et al.*, *Mech. Dev.* **108**, 121-133 (2001)

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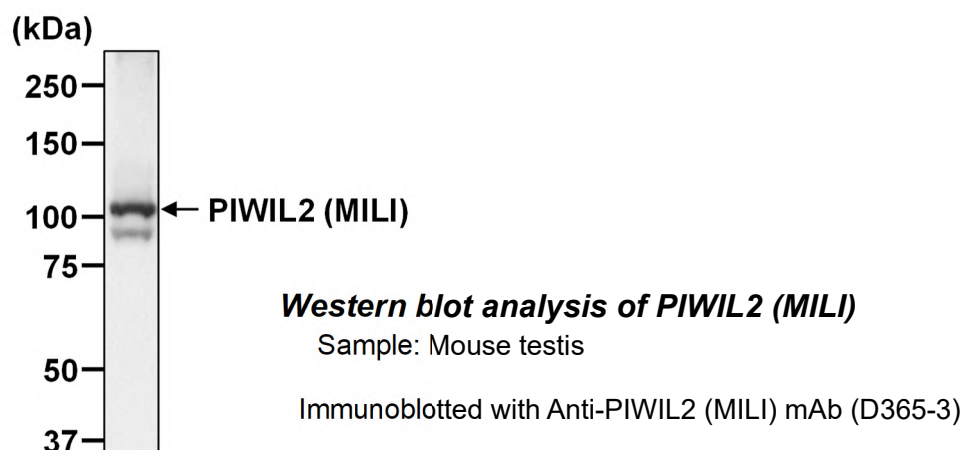
RELATED PRODUCTS

D365-3 Anti-PIWIL2 (MILI) mAb (3G5-E6)
RN010MW Anti-PIWIL1 (MIWI) mAb (2D9)
PM043 Anti-PIWIL2 (MILI) (Mouse) pAb
PM044 Anti-PIWIL2 (MILI) (Mouse) pAb
D363-3 Anti-Ly6k (Mouse) mAb (mk34)
D362-3 Anti-Tex101 (Mouse) mAb (mTX5.2)
D357-3 Anti-Ace (CD143) (Mouse) mAb (1D5)
M207-3 Anti-MitoPLD (Pld6) mAb (26C46-6)
D356-3 Anti-Jmjd1c (Mouse) mAb (13B)
M075-3 Mouse IgG1 (isotype control) (2E12)

SDS-PAGE & Western blotting

- 1) Load 50 µg of tissue lysate per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 2) 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.
- 3) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) overnight at 4°C.
- 4) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
- 6) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
- 7) Wash the membrane with PBS-T (3 times for 5 min.).
- 8) 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.
- 9) Expose to an X-ray film for 3 min. in a dark room. Develop the film as usual. The condition for exposure and development may vary.

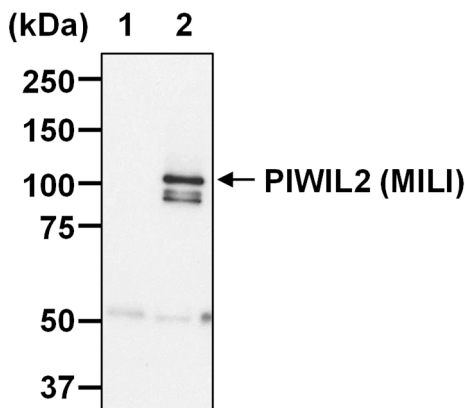
(Positive control for Western blotting; Mouse testis)



Immunoprecipitation

- 1) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 200 μ L of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] containing appropriate protease inhibitors with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
- 2) Wash the beads 3 times with 1 mL of IP buffer.
- 3) Add 30 mg of tissue lysate and 200 μ L of IP buffer containing appropriate protein inhibitors. Incubate with gentle agitation for 1 hr. at room temperature.
- 4) Wash the beads 6 times with 1 mL of Extraction buffer [150 mM Na₂HPO₄ (pH7.4), 60 mM *n*-octyl- β -D-glucopyranoside, 10 mM D-gluconic acid lactone, 20 mM EDTA].
- 5) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 6) Load 10 μ L per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 7) 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.
- 8) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) overnight at 4°C.
- 9) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
- 11) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
- 12) Wash the membrane with PBS-T (3 times for 5 min.).
- 13) 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.
- 14) Expose to an X-ray film for 3 min. in a dark room. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse testis)



Immunoprecipitation of PIWIL2 (MILI)

Sample: Mouse testis

Lane 1: Mouse IgG1 (isotype control) (M075-3)

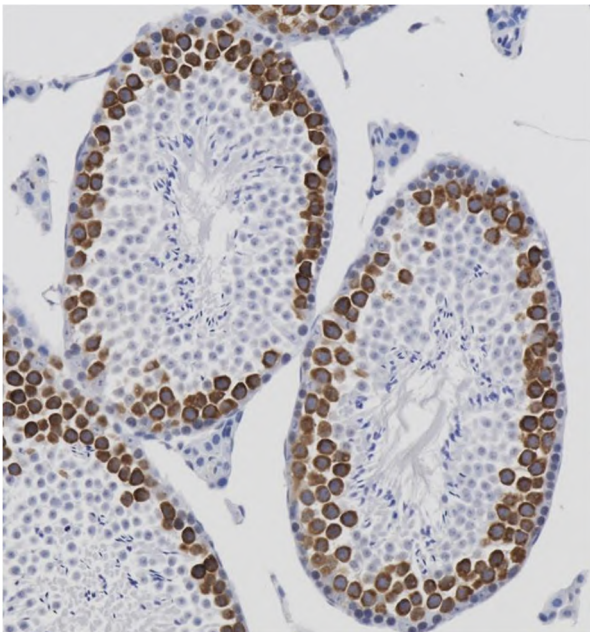
Lane 2: Anti-PIWIL2 (MILI) mAb (D365-3)

Immunoblotted with Anti-PIWIL2 (MILI) mAb (D365-3)

Immunohistochemical staining for paraffin embedded section

- 1) Deparaffinize the section with Xylene 3 times for 3 min. each.
- 2) Immerse the slide with Ethanol 3 times for 3 min. each.
- 3) Immerse the slide with PBS 3 time for 3 min.
- 4) Remove the slide from PBS and heat-treat with 10 mM citrate buffer (pH 6.0) for 2 times for 10 min. using microwave oven.
- 5) Let the slide cool down at room temperature in citrate buffer.
- 6) Remove the slide from citrate buffer and inactivate endogenous peroxidase with 3% H₂O₂ in PBS for 10 min.
- 7) Wash the slide 3 times in PBS for 5 min. each.
- 8) Immerse the slide in Blocking buffer [20 mM HEPES, 1% BSA, 135 mM NaCl] for 5 min. at room temperature to block non-specific staining.
- 9) Incubate the section with the primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 10) Wash the slides 2 times in PBS for 5 min. each.
- 11) Incubate the section with Histostar (Ms + Rb) (MBL; code no. 8460) for 30 min. at room temperature.
- 12) Wash the slides 3 times in PBS for 5 min. each.
- 13) Visualize by reacting for 1 min. with Histostar DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 14) Wash the slides 3 times in PBS for 5 min. each.
- 15) Counterstain in hematoxylin for 5 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 testis)



Immunohistochemical detection of PIWIL2 (MILI) in mouse testis

Brown: Anti-PIWIL2 (MILI) mAb (D365-3)
Blue: Hematoxylin