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MBL
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POLYCLONAL ANTIBODY

Anti-PIWIL2 (MILI) (Mouse) pAb

Code No.	Quantity	Form
PM044	100 µL	Affinity Purified

BACKGROUND: The Piwi/Argonaute protein family plays important roles in stem-cell self-renewal, RNA silencing and translational regulation in various organisms. The family members are defined by conserved both PAZ (Piwi Argonaut and Zwille) and Piwi domains participate in RNA binding. Piwi-interacting RNA (piRNA) is a class of small RNA molecules that is expressed uniquely in mammalian testes and forms RNA-protein complexes with Piwi proteins. These small regulatory RNAs have been implicated in spermatogenesis, repression of retrotransposon transposition in germline cells, epigenetic regulation and positive regulation of translation and mRNA stability. MIWI (PIWIL1) and MILI (PIWIL2) are mouse homologues of Piwi. MILI is expressed from primordial germ cells (PGC) in embryo, whereas MIWI is detected later from the mid-pachytene stage to early round spermatids. Their expression overlaps in the mid-pachytene and early round spermatid stage. The expression patterns and knockout phenotypes of mammalian Piwi members indicated MILI is an essential factor for meiotic differentiation during spermatogenesis. It was also reported that MILI (PIWIL2) is widely expressed in various tumors and acts as an oncogene by inhibiting apoptosis and promoting proliferation through the activation of Stat3/Bcl-xL pathway.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the synthetic peptide, VRKDREEPRSSLPDPS, corresponding to mouse PIWIL2 (107-122 aa).

FORMULATION: 100 µL volume of 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.

REACTIVITY: This antibody reacts with MILI on Western blotting and Immunoprecipitation.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue	Not Tested	testis	Not Tested
Reactivity on WB		+	

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system.
Immunoprecipitation; 1 µL/sample
Immunohistochemistry; Not recommended*
*PM043 is suitable for this application.
Immunocytochemistry; Not tested
Flow cytometry; Not tested
RNP immunoprecipitation (RIP); Reference 2)-4)

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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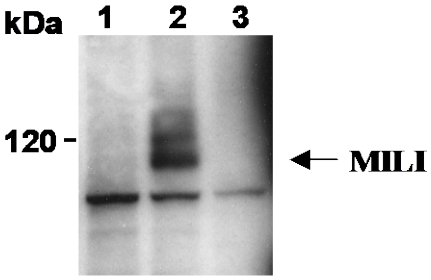
REFERENCES:

1) Shiromoto, Y., *et al*., *RNA* **19,** 803-810 (2013)
2) Kuramochi-Miyagawa, S., *et al*., *Genes Dev*. **24**, 887-892 (2010) [RIP]
3) Kojima, K., *et al*., *Genes Cells.* **14**, 1155-1165 (2009) [WB, IP, RIP]
4) Kuramochi-Miyagawa, S., *et al*., *Genes Dev*. **22**, 908-917 (2008) [RIP]
5) Aravin, A. A., *et al., Science* **316**, 744-747 (2007)
6) Aravin, A., *et al., Nature* **442**, 203-207 (2006)
7) Kuramochi-Miyagawa, S., *et al., Development* **131**, 839-849 (2004)
8) Kuramochi-Miyagawa, S., *et al., Mech. Dev*. **108**, 121-133 (2001)

This antibody is used in reference number 1)-4).



Western blot analysis of MILI expression in MILI-KO mouse testis (1), WT mouse testis (2) and mouse spleen (3) using PM044.
This data was kindly provided by Dr. Satomi Kuramochi-Miyagawa. (Graduate school of Frontier Biosciences, Osaka University)

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

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4ºC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
5) 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 manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk/TBS-T (0.05% Tween-20 in TBS, pH 7.2) for 1 hour at room temperature, or overnight at 4ºC.
7) Incubate the membrane with primary antibody diluted with TBS-T, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with TBS-T (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in TBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with TBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Mouse testis)

Immunoprecipitation of MILI on mouse testis with PM044 (left) or normal rabbit IgG (right). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM044.*
This data was kindly provided by Dr. Satomi Kuramochi-Miyagawa. (Graduate school of Frontier Biosciences, Osaka University)

Immunoprecipitation

1) Wash the sample 2 times with PBS and homogenate with cold Lysis buffer (50 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 0.1% NP-40, 5 mM EDTA, 10% glycerol) containing appropriate protease inhibitors.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4°C with rotating for 30-60 minutes.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tube.
5) Add the normal rabbit IgG or anti-MILI antibody at the amount of as suggest in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 90-120 minutes at 4°C.
6) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
7) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
8) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Mouse testis)

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