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

Anti-Ly6k (Mouse) mAb

CODE No. D363-3

CLONALITY Monoclonal
CLONE mk34
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Mouse testicular water-insoluble fraction
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 µg/mL for chemiluminescence detection system (non-reducing condition)
Immunoprecipitation	15 µg/sample
Flow cytometry	1 µg/mL

APPLICATION-REPORTED

Immunohistochemistry	Reference 1) and 2)

SPECIES CROSS REACTIVITY on WB

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

Entrez Gene ID 76486 (Mouse)

REFERENCES

1) Endo, S., *et al.*, *Sci. Rep.* **6**, 23616 (2016)
2) Maruyama, M., *et al.*, *Biochem. Biophys. Res. Commun.* **402**, 75–81 (2010)
3) Yoshitake, H., *et al.*, *Biochem. Biophys. Res. Commun.* **372**, 277-282 (2008)
4) Tsukamoto, H., *et al.*, *Biochem. Biophys. Res. Commun.* **345**, 229-238 (2006)

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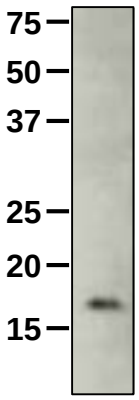
RELATED PRODUCTS

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)
RN010MW	Anti-PIWIL1 (MIWI) mAb (2D9)
PM043	Anti-PIWIL2 (MILI) (Mouse) pAb
PM044	Anti-PIWIL2 (MILI) (Mouse) pAb
D356-3	Anti-Jmjd1c (Mouse) mAb (13B)
M081-3	Rat IgG2a (isotype control) (2H3)

SDS-PAGE & Western blotting

1) Mix 20 mg of mouse testis with 1 mL of Laemmli's sample buffer (non-reducing condition), then sonicate for 10 sec. Incubate on ice for 10 min.
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 supernatant for 3 min. and centrifuge.
1) Load 20 μL of the supernatant per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 190 mA 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) for 1 hr. at room temperature.
4) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
5) 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.)
6) Wash the membrane with PBS-T (3 times for 5 min.).
7) Incubate the membrane with HRP-conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (3 times for 5 min.).
9) Wash the membrane 1 time for 2 min. with PBS.
10) 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.
11) 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)



Western blot analysis of mouse Ly6k

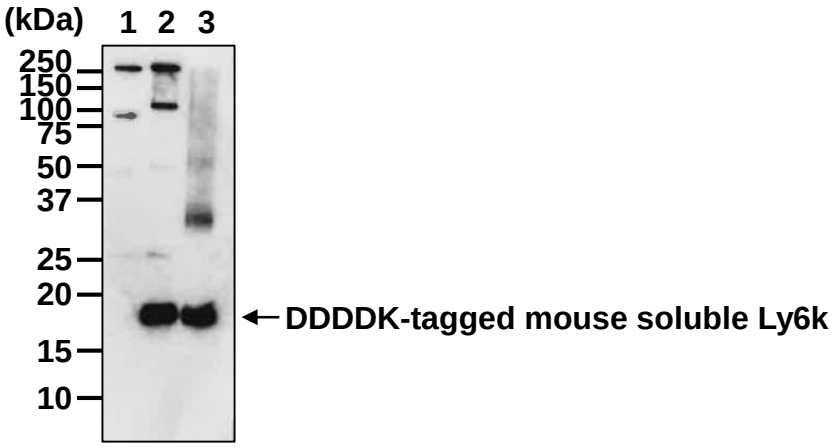
Sample: Mouse testis

Immunoblotted with Anti-Ly6k (Mouse) mAb (D363-3)

Immunoprecipitation

1) Mix 20 μL of 50% protein G agarose beads slurry resuspended in 400 μL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
2) Wash the beads 1 time with 1 mL of IP buffer.
3) Add 1 mL of cuture supernatant, then incubate with gentle agitation for 1 hr. at room temperature.
4) Wash the beads 5 times with 1 mL of Extraction buffer [50 mM Tris-HCl (pH7.5), 150 mM NaCl, 0.05% NP-40].
5) Resuspend the beads in 25 μL of Laemmli's sample buffer (non-reducing condition), boil for 3 min. and centrifuge.
6) Load 10 μL per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
7) 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.
8) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
10) Incubate the membrane with 0.1 μg/mL of Anti-DDDDK-tag mAb (MBL; code no. M185-3) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
11) Wash the membrane with PBS-T (3 times for 5 min.).
12) Incubate the membrane with the 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.
13) Wash the membrane with PBS-T (3 times for 5 min.).
14) 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.
15) 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 control for Immunoprecipitation; Culture supernantant of transfectant)



Immunoprecipitation of mouse soluble Ly6k

Sample: Culture supernatant of DDDDK-tagged mouse soluble Ly6k/CHO
Lane 1: Rat IgG2a (isotype control) (M081-3)
Lane 2: Anti-Ly6k (Mouse) mAb (D363-3)
Lane 3: Anti-DDDDK-tag mAb (M185-3)

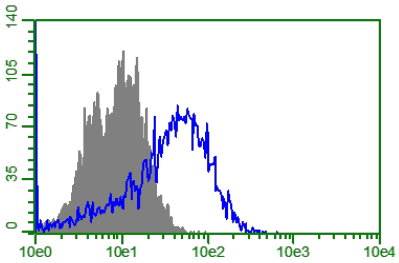
Immunoblotted with Anti-DDDDK-tag mAb (M185-3)

Sample was kindly provided by Dr. Hiroki Tsukamoto. (Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University)

Flow cytometric analysis

1) Wash the cells (1.4 x 10^6 cells/sample) 1 time with 5 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 10 µL of normal goat serum to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
3) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with washing buffer.
4) Mix well and incubate for 30 min. at room temperature.
5) Wash the cells 1 time with 1 mL of washing buffer.
6) Add the secondary antibody diluted with washing buffer.
7) Mix well and incubate for 30 min. at room temperature.
8) Wash the cells 1 time with 1 mL of washing buffer.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)



Flowcytometric detection of mouse Ly6k

Cells: Mouse Ly6k/293T
Open: Anti-Ly6k (Mouse) mAb (D363-3)
Closed: Rat IgG2a (isotype control) (M081-3)

Sample was kindly provided by Dr. Hiroki Tsukamoto. (Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University)