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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY **Mitochondrial Marker**

Anti-COX4 pAb

Code No.	Quantity	Form
PM063	100 µL	Affinity Purified

BACKGROUND: The mitochondria are membrane-enclosed organelle found in most eukaryotic cells. The mitochondria serve many general functions, including production of ATP, signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth.
COX4 is the nuclear-encoded subunit IV isoform 1 of the mitochondrial respiratory chain enzyme. This antibody is an effective mitochondrial marker, because COX4 is constantly expressed at high level in mitochondria.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with synthetic peptide corresponding to N-terminus of human COX4.

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 human COX4 on Western blotting, Immunoprecipitation, and Immunocytochemistry. The reactivity to mouse, rat, hamster and chicken COX4 was confirmed by Western blotting.

APPLICATIONS:
Western blotting; 1:1,000 for a chemiluminescence detection system
Immunoprecipitation; 2 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 1:500
Flow cytometry; Not tested

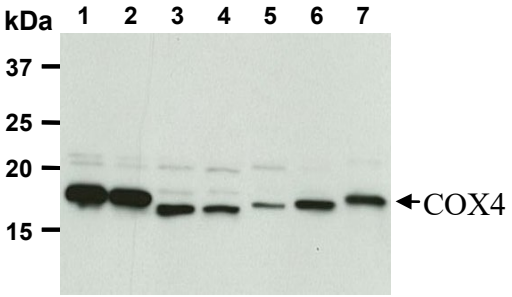
Detailed procedures are provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster	Chicken
Cells	HeLa 293T	NIH/3T3 MEF	Rat1	CHO	MuH1
Reactivity on WB	+	+	+	+	+

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Van Kuilenburg, A. B., *et al.*, *Biochim. Biophys. Acta* **1119**, 218-224 (1992)
2) Zeviani, M., *et al.*, *Gene* **55**, 205-217 (1987)



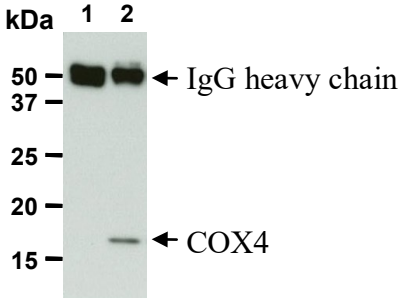
Western blot analysis of COX4 in HeLa (1), 293T (2), NIH/3T3 (3), MEF (4), Rat1 (5), CHO (6) and MuH1 (7) using PM063.

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

PROTOCOLS:
SDS-PAGE & Western Blotting
1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) 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 manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).

7) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 1 minute. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, NIH/3T3, MEF, Rat1, CHO and MuH1)



Immunoprecipitation of COX4 from HeLa with normal rabbit IgG (1) or PM063 (2). *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with PM063.*

Immunoprecipitation

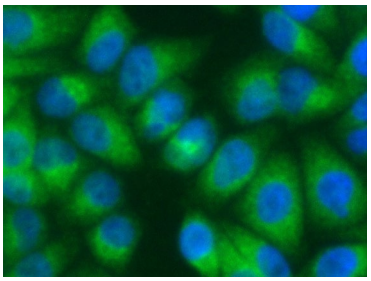
1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4ºC with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 20 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at room temperature. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at room temperature.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10^4 cells of HeLa cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
4) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.2% TritonX-100 for 10 minutes at room temperature.
6) The glass slide was washed 2 times with PBS.
7) Add the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) The glass slide was washed 2 times with PBS.
9) Add 100 µL of 1:500 Alexa Fluor®488 conjugated anti-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 2times with PBS.
11) Counter stain with DAPI for 5 minutes at room temperature.
12) The glass slide was washed 2 times with PBS.
13) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)



Immunocytochemical detection of COX4 in HeLa using PM063.
Green: anti-COX4
Blue: DAPI counter stain

RELATED PRODUCTS:

Please visit our web site https://ruo.mbl.co.jp/