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

MONOCLONAL ANTIBODY

Anti-NADPH-Flavin Reductase

Code No.	Clone	Subclass	Quantity	Form
M064-3	2C10	Mouse IgG1	100 µg	Lyophilized

BACKGROUND: Methemoglobinemia is a blood disease originated from oxidization of hemoglobin-iron ions (Fe(II) → Fe(III)). Oxidized hemoglobin (methemoglobin) which can not carry oxygen, is rereduced by enzyme named methemoglobin reductase or administrated flavin or methylene blue.

NADPH-flavin reductase (FLR) is a 21 kDa protein that catalyzes electron transfer from mainly NADPH to flavins (FMN, FAD) and a variety of other electron acceptors including methylene blue, 2,6-dichlorophenolindophenol (DCIP), and pyrroloquinoline quinone (PQQ). FLR was originally identified as a candidate of NADPH dependent methemoglobin reductase. However it was found that, though this enzyme reduces methemoglobin through reducing flavin or methylene blue, it does not reduce methemoglobin directly under physiological conditions.

It was also reported that human FLR is identical with human biliverdin-IXβ-reductase (BLVR-B) which participate in hem catabolism. Hem, which is a component of hemoglobin is metabolized to a biliverdin by hemoxygenase. Biliverdin-IXβ, which is one of isomers of biliverdin is then reduced to bilirubin-IXβ by BLVR-B. Though physiological functions for FLR have not been clear, one of the important functions may be the reduction of biliverdin-IXβ.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (2C10) was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the recombinant full-length human NADPH-Flavin reductase.

FORMULATION: This antibody is lyophilized form. Prepare a stock solution by dissolving the lyophilized antibody in 100 µL of distilled water. After reconstitution, the IgG concentration should be 1 mg/mL in PBS (pH 7.2) /1% sucrose. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of shipment when stored at 4°C. After reconstitution, avoid repeated freezing and thawing. For storage, prepare appropriate aliquots and freeze them at -20°C.

REACTIVITY: This antibody reacts with FLR on Western blotting.

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APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Monky
Cells	ZR-75-1, U937, Raji, HeLa	Ba/F3	PC12	COS-7
Reactivity on WB	+	weak	+	+

REFERENCES:
1) Shalloe, F., *et al*., *Biochem. J*. **316**, 385-387 (1996)
2) Komuro, A., *et al*., *Biol. Pharm. Bull*. **19**, 796-804 (1996)
3) Yamaguchi, T., *et al*., *J. Biol. Chem*. **269**, 24343-24348 (1994)
4) Chikuba, K., *et al*., *Biochem. Biophys. Res. Commun*. **198**, 1170-1176 (1994)
5) Yamaguchi, T., *et al*., *Biochem. Biophys. Res. Commun*. **197**, 1518-1523 (1993)
6) Yubisui, T., *et al*., *Biochem. Int*. **15**, 1-8 (1987)

PROTOCOLS:
SDS-PAGE & Western Blotting
1) Wash the $1x10^7$ cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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 manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, 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.)

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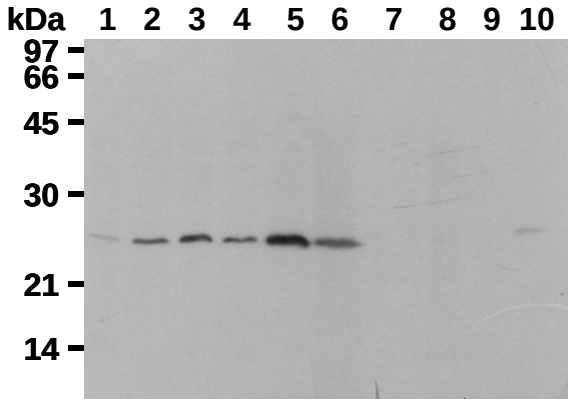
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, HeLa, MRC-5, ZR-75-1, U937, Ba/F3)



Western blot analysis of NADPH-Flavin Reductase expression in Jurkat (1), Raji (2), HeLa (3), MRC-5 (4), ZR-75-1 (5), U937 (6), NIH/3T3 (7), WR19L (8), P19 (9) and Ba/F3 (10) using M064-3.