

MONOCLONAL ANTIBODY

Anti-Flavocytochrome b₅₅₈ (Human) mAb-FITC

Code No.	Clone	Subclass	Quantity	Concentration
D162-4	7D5	Mouse IgG1	100 µL	500 µg/mL

BACKGROUND: The NADPH oxidase is a multicomponent enzyme that transfers electrons from NADPH to O₂ to generate superoxide (O₂⁻), a key part of the phagocytic or neutrophilic respiratory burst response. Flavocytochrome b₅₅₈ is the catalytic component of the phagocyte NADPH oxidase. It is a transmembrane heterodimer composed of a large glycoprotein, gp91^{phox} (PHagocyte OXidase) and a smaller protein, p22^{phox}. Upon cell stimulation, flavocytochrome b₅₅₈ assembles with p67^{phox}, p47^{phox}, and the GTP-binding protein Rac and becomes activated to generate O₂⁻. Mutations in gp91^{phox}, p22^{phox}, or other components of the NADPH oxidase can result in chronic granulomatous disease, which is associated with significant morbidity and mortality due to a predisposition to recurrent bacterial and fungal infections.

SOURCE: This antibody was purified from hybridoma (clone 7D5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Balb/c mouse splenocyte immunized with the human cytochrome b rich fraction.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.09% NaN₃.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with Flavocytochrome b₅₅₈ on flow cytometry.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunocytochemistry; Not tested
- Immunohistochemistry; Not tested
- Flow Cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

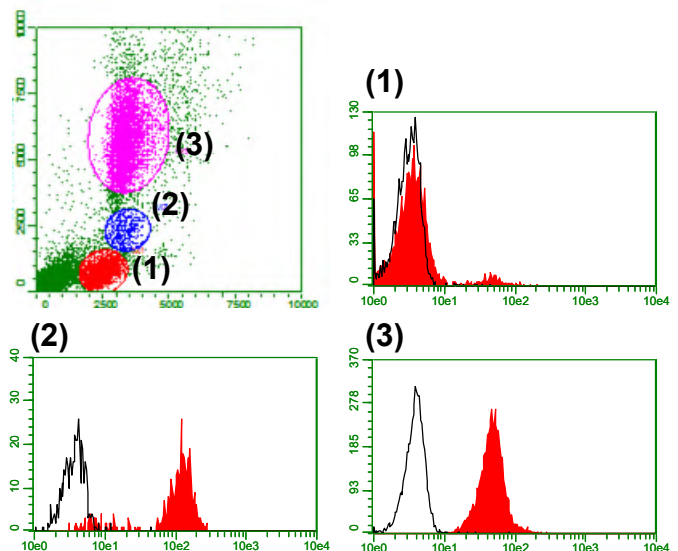
Species	Human	Mouse	Rat
Cells	Lymphocyte Monocyte Granulocyte	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:

For research use only. Not for clinical diagnosis.

REFERENCES:

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- 9) Burritt, J. B., *et al.*, *J. Biol. Chem.* **276**, 2053-2061 (2001)
- 10) Yu, L., *et al.*, *Blood* **94**, 2497-2504 (1999)
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- 14) Nakamura, M., *et al.*, *Blood* **69**, 1404-1408 (1987)



Flow cytometric analysis of Flavocytochrome b₅₅₈ expression on Lymphocytes (1), Monocytes (2) and Granulocytes (3). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D162-4 to the cells.

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

PROTOCOL:

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all step described below.

- 1) Add 20 μ L of the primary antibody as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃] into each tube.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

- 2) Add 100 μ L of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
- 3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B using the procedure recommended in the respective package inserts.
- 5) Add 1 mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for flow cytometry: Human lymphocyte, monocyte and granulocyte)

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