

MONOCLONAL ANTIBODY

Anti-Flavocytochrome b₅₅₈ (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
D162-5	7D5	Mouse IgG1	1 mL (50 tests)

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 tests in 1 mL volume of PBS containing 1% BSA and 0.1% ProClin 150.

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

REACTIVITY: This antibody recognizes the extracellular peptide portion of primate gp91^{phox} of the human Flavocytochrome b₅₅₈ on Flow cytometry.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunocytochemistry; Not tested
- Immunohistochemistry; Not tested
- Flow cytometry; 20 µL (ready for use)

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

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

*Clone 7D5 reacts with monkey but not mouse gp91^{phox} 13).

REFERENCES:

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- 11) Carlyon, J. A., *et al.*, *J. Immunol.* **169**, 7009-7018 (2002)
- 12) Morgan, D., *et al.*, *J. Gen. Physiol.* **119**, 571-580 (2002)
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Clone 7D5 is used in these references.

PROTOCOL:

Flow cytometric analysis for whole blood cells

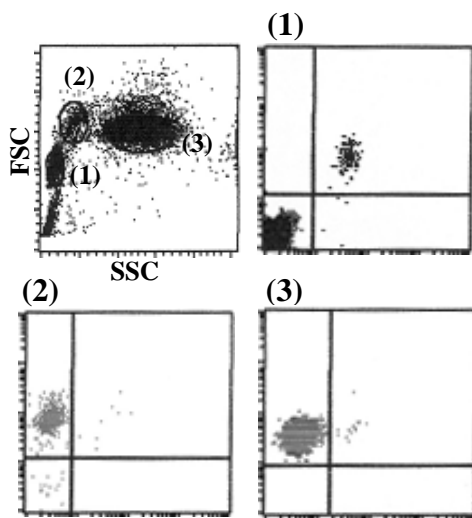
We usually use Falcon tubes or equivalents as reaction tubes for all step described below.

- 1) Add the primary antibody as suggested in the **APPLICATIONS** into each tube.
- 2) Add 50 µL 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 [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃] followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.

*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.

- 4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry: Granulocyte, Monocyte and Lymphocyte)



Flow cytometric analysis of Flavocytochrome b_{558} expression on Lymphocyte (1), Monocyte (2) and Granulocyte (3). The staining intensity of D162-5 is shown in the vertical axis with CD19 staining on the horizontal axis.