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MONOCLONAL ANTIBODY

Anti-Flavocytochrome b_{558} (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 O_2 to generate superoxide (O_2^-), a key part of the phagocytic or neutrophilic respiratory burst response. Flavocytochrome b_{558} 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 b_{558} assembles with $p67^{phox}$, $p47^{phox}$, and the GTP-binding protein Rac and becomes activated to generate O_2^-. 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% NaN_3.
*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.

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 b_{558} 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:
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REFERENCES:
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4) Makni-Maalej, K., *et al., J. Immunol.* **189**, 4657-4665 (2012) [FCM]
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12) Radeke, H. H., et al., *J. Biol. Chem.* **266**, 21025-21029 (1991)
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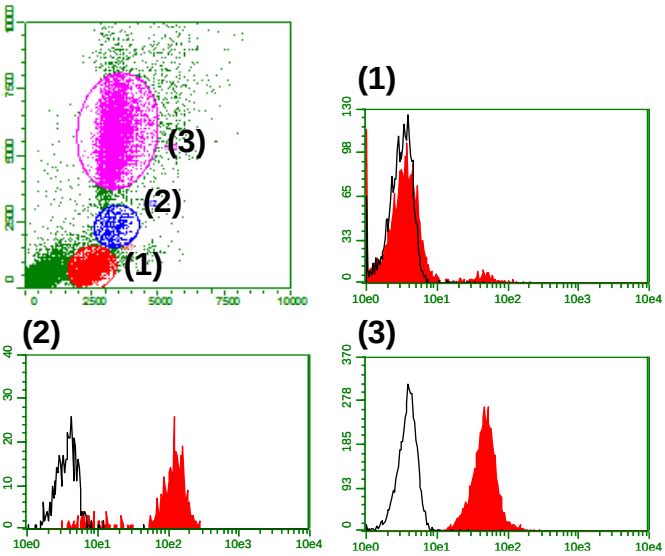
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Flow cytometric analysis of Flavocytochrome b_{558} 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.*

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 µL of the primary antibody as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] 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 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 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 H_2O 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: Human lymphocyte, monocyte and granulocyte)

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