

MONOCLONAL ANTIBODY

# Anti-Flavocytochrome b<sub>558</sub> (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D162-3	7D5	Mouse IgG1	100 µL	1 mg/mL

**BACKGROUND:** The NADPH oxidase is a multicomponent enzyme that transfers electrons from NADPH to O<sub>2</sub> to generate superoxide (O<sub>2</sub><sup>-</sup>), a key part of the phagocytic or neutrophilic respiratory burst response. Flavocytochrome b<sub>558</sub> is the catalytic component of the phagocyte NADPH oxidase. It is a transmembrane heterodimer composed of a large glycoprotein, gp91<sup>phox</sup> (PHagocyte OXidase) and a smaller protein, p22<sup>phox</sup>. Upon cell stimulation, flavocytochrome b<sub>558</sub> assembles with p67<sup>phox</sup>, p47<sup>phox</sup>, and the GTP-binding protein Rac and becomes activated to generate O<sub>2</sub><sup>-</sup>. Mutations in gp91<sup>phox</sup>, p22<sup>phox</sup>, 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:** 100 µg IgG in 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 Flavocytochrome b<sub>558</sub> flow cytometry.

**APPLICATIONS:**

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunocytochemistry; Not tested
- Immunohistochemistry; Not tested
- Flow cytometry; 5 µg/mL (final concentration)

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

**INTENDED USE:**

For research use only. Not for clinical diagnosis.

**RELATED PRODUCTS:**

- D162-4 Anti-Flavocytochrome b<sub>558</sub> (Human)-mAb-FITC
- D162-5 Anti-Flavocytochrome b<sub>558</sub> (Human)-mAb-PE

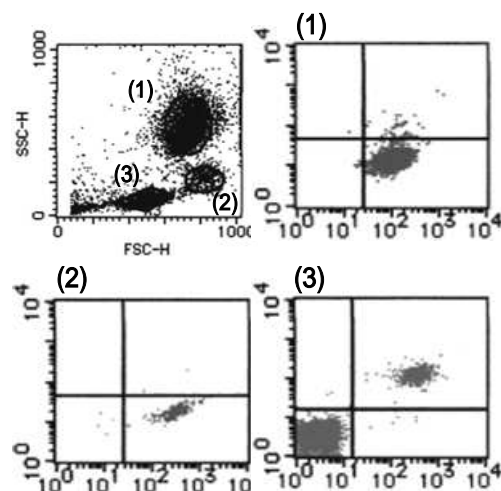
**SPECIES CROSS REACTIVITY:**

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

**REFERENCES:**

- 1) Baba, L. A., *et al.*, *J. Clin. Immunol.* **34**, 452-458 (2014) [FCM]
- 2) Debeurme, F., *et al.*, *J. Biol. Chem.* **285**, 33197-33208 (2010) [FCM]
- 3) Li, X. J., *et al.*, *J. Leukoc. Biol.* **81**, 238-249 (2007) [FCM]
- 4) Yamauchi A, *et al.*, *Microbiol. Immunol.* **45**: 249-57 (2001)
- 5) Burritt, J. B., *et al.*, *J. Biol. Chem.* **276**: 2053-2061 (2001)
- 6) Yu, L., *et al.*, *Blood* **94**: 2497-2504 (1999)
- 7) Yu, L., *et al.*, *PNAS* **95**: 7993-7998 (1998)
- 8) Radeke, H. H., *et al.*, *J. Biol. Chem.* **266**: 21025-21029 (1991)
- 9) Verhoeven, A., *et al.*, *Blood* **73**: 1686-1694 (1989)
- 10) Nakamura, M., *et al.*, *Blood* **69**: 1404-1408 (1987)

Clone 7D5 is used in these references.



**Flow cytometric analysis of Flavocytochrome b<sub>558</sub> expression on Granulocytes (1), Monocytes (2) and Lymphocytes (3). The staining intensity of D162-3 is shown in the horizontal axis with CD19-FITC staining on the vertical axis.**

## **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  $\mu$ 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%  $\text{NaN}_3$ ] into each tube.
- 2) Add 50  $\mu$ 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) Add 20  $\mu$ L of PE conjugated anti-mouse IgG (H+L) antibody diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 6) Add 20  $\mu$ L of FITC conjugated anti-human CD 19 antibody. Mix well and incubate for 15 minutes at room temperature.
- 7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B using the procedure recommended in the respective package inserts.
- 9) Add 1 mL of  $\text{H}_2\text{O}$  to each tube and incubate for 10 minutes at room temperature.
- 10) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 11) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 12) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer

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