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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Flavocytochrome b_{558} (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 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 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN3.
*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 recognizes the extracellular peptide portion of primate $gp91^{phox}$ of the human Flavocytochrome b_{558} 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:
1) Zou, J., *et al.*, *Blood* **117**, 5561-5572 (2011) [FCM]
2) Li, X. J., *et al.*, *J. Leukoc. Biol.* **81**, 238-49 (2007)
3) Reumaux, D., *et al.*, *J. Leukoc. Biol.* **80**, 1424-1433 (2006)
4) Taylor, R. M., *et al.*, *J. Biol. Chem.* **281**, 37045-37056 (2006)
5) Zhu, Y., *et al.*, *J. Biol. Chem.* **281**, 30336-30346 (2006)
6) Li, X. J., *et al.*, *J. Biol. Chem.* **280**, 14962-14973 (2005)
7) Allen, L. A. H., *et al.*, *J. Immunol.* **174**, 3658-3667 (2005)
8) Carlyon, J. A., *et al.*, *Infect. Immun.* **72**, 4772-4783 (2004)
9) Brenner, S., *et al.*, *Blood* **102**, 2789-2797 (2003)
10) Burritt, J. B., *et al.*, *J. Immunol.* **170**, 6082-6089 (2003)
11) Carlyon, J. A., *et al.*, *J. Immunol.* **169**, 7009-7018 (2002)
12) Morgan, D., *et al.*, *J. Gen. Physiol.* **119**, 571-580 (2002)
13) Yamauchi, A., *et al.*, *Microbiol. Immunol.* **45**, 249-57 (2001)
14) Burritt, J. B., *et al.*, *J. Biol. Chem.* **276**, 2053-2061 (2001)
15) Yu, L., *et al.*, *Blood* **94**, 2497-2504 (1999)
16) Yu, L., *et al.*, *Proc. Natl. Acad. Sci. USA* **95**, 7993–7998 (1998)
17) Radeke, H. H., *et al.*, *J. Biol. Chem.* **266**, 21025-21029 (1991)
18) Verhoeven, A., *et al.*, *Blood* **73**, 1686-1694 (1989)
19) Nakamura, M., *et al.*, *Blood* **69**,1404-1408 (1987)

Clone 7D5 is used in these references.

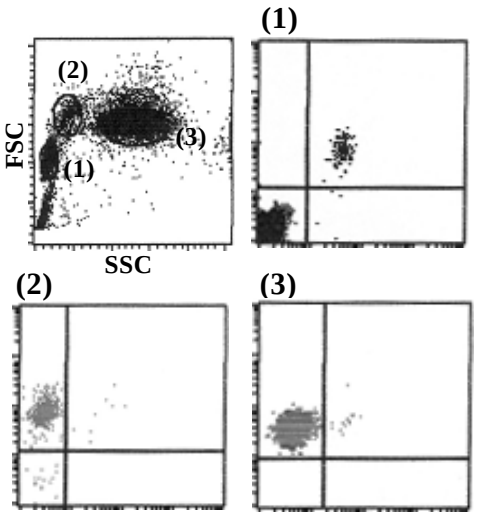
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 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% NaN_3] 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 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: 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.*

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.