Product datasheet for AM26496RP-N

CYBA Mouse Monoclonal Antibody [Clone ID: 7D5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	7D5
Applications:	FC
Recommended Dilution:	Flow cytometry: 20 µl (ready for use). For details see protocol below.
Reactivity:	Human, Monkey
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Human cytochrome b-rich fraction
Specificity:	This antibody recognizes the extracellular peptide portion of primate gp91phox of the Flavocytochrome b558.
Formulation:	PBS Label: PE State: Liquid Ig fraction Stabilizer: 1% BSA Preservative: 0.09% NaN3
Purification:	Protein A agarose
Conjugation:	PE
Storage:	Store at 2-8 °C.
Stability:	Shelf life: one year from despatch.
Gene Name:	cytochrome b-245 alpha chain
Database Link:	Entrez Gene 1535 Human P13498

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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, gp91phox (PHagocyte OXidase) and a smaller protein, p22phox. Upon cell stimulation, flavocytochrome b558 assembles with p67phox, p47phox, and the GTP-binding protein Rac and becomes activated to generate O2 -. Mutations in gp91phox, p22phox, 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.

Synonyms: CYBA, p22-phox, Cytochrome b558 subunit alpha

**Note:** This product was originally produced by MBL International.

Protocol:
Flow cytometric analysis for whole blood cells
We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.
1) Add 20 µL of the PE labeled anti-Flavocytochrome b558 monoclonal antibody (7D5) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3] 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 oC).
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 (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, Lymphocyte)

Product images:
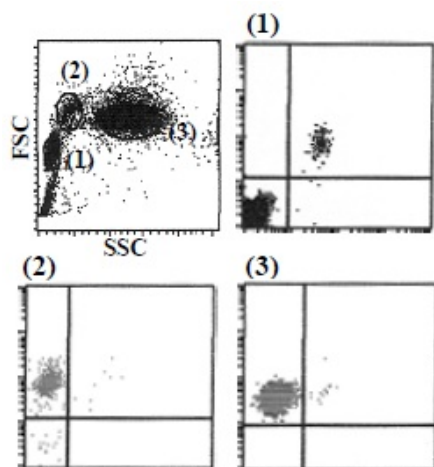
Flow cytometric analysis of Flavocytochrome b558 expression on Lymphocyte (1), Monocyte (2) and Granulocyte (3). The staining intensity of AM26496RP-N is shown in the vertical axis with CD19 staining on the horizontal axis.