

Technical Data Sheet

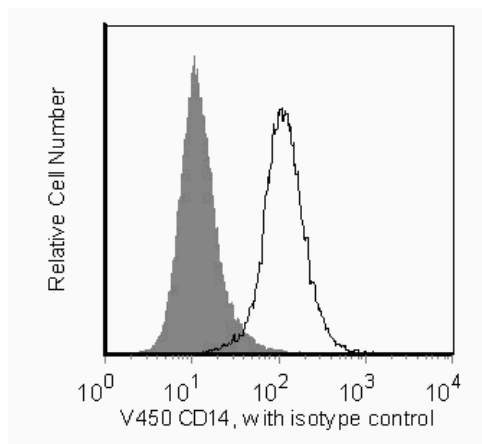
V450 Rat Anti-Mouse CD14**Product Information**

Material Number:	560639
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	rmC5-3
Immunogen:	Recombinant Mouse CD14
Isotype:	Rat (LOU) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The rmC5-3 antibody reacts with residues 308-322 of the hydrophilic region of mouse CD14. CD14 is a 53-55 kDa glycosyl phosphatidyl inositol (GPI)-linked glycoprotein belonging to the leucine-rich glycoprotein repeat superfamily of cell-surface proteins. It is a receptor for the complex of lipopolysaccharide (LPS or endotoxin, from gram-negative bacteria) with LPS-binding protein (LBP, a plasma protein). It is involved in the development of endotoxic shock and LPS-stimulated bone resorption, and promotes, possibly indirectly, bacterial dissemination. Flow cytometric analysis demonstrates that rmC5-3 antibody stains J774A.1 (mouse macrophage line), WEHI-265.1 (mouse monocytic line), peritoneal resident macrophages, Kupffer cells, and cultured bone marrow-derived macrophages and dendritic cells, but not unstimulated splenic macrophages, dendritic cells, neutrophils, or blood monocytes. This staining pattern is similar to that of the alternate anti-mouse CD14 mAb 4C1/CD14, which recognizes a different CD14 epitope, and differs from that of the human, where CD14 expression is characteristic of circulating monocytes and neutrophils. Therefore, data suggests that CD14 expression by leukocyte populations may differ in mice and humans. Peritoneal cells from naive mice, 3-day thioglycollate-elicited peritoneal exudate, as well as 4-hour LPS-activated peritoneal cells, contain a population of Mac-1 (CD11b)-high cells which double-stain with rmC5-3 antibody. Levels of CD14 expression on Kupffer cells and bone marrow-derived macrophages and dendritic cells of LPS-sensitive mice are increased by in vivo and in vitro LPS treatments, an effect which may be mediated by TNF-α. Preliminary evidence suggests that CD14 may be up-regulated on mouse blood neutrophils. In agreement with the observations that CD14 is shed from activated human and mouse monocytes, rmC5-3 mAb detects soluble CD14 in the serum of LPS-treated mice in a time-dependent manner.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Flow cytometric analysis of CD14 on J774A.1 cells.
J774A.1 cells (Mouse monocyte/macrophage cells; ATCC TIB-67) were stained either with a BD Horizon™ V450 Rat IgG1, κ isotype control (shaded) or with the BD Horizon™ V450 Rat Anti-Mouse CD14 antibody (unshaded). Histograms were derived from gated events based on light scattering characteristics for J774A.1 cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

Flow Cytometry: Investigators should note that Mouse BD Fc Block™ purified rat anti-CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) and antibodies of the rat IgG2b isotype may potentially interfere with the reactivity of the BD Horizon™ V450 Rat Anti-Mouse CD14 antibody (clone rmC5-3) in a concentration-dependent manner. For alternative methods for inhibition of non-specific reactivity, investigators may find the use of purified mouse IgG at a 10-100-fold excess to be more appropriate.

Suggested Companion Products

Catalog Number	Name	Size	Clone
560535	V450 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

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