Technical Data Sheet

Purified Rat Anti-Mouse CD14

Product Information

Material Number:	**553738**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	rmC5-3
Immunogen:	Recombinant Mouse CD14
Isotype:	Rat (LOU) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤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.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Flow cytometry	Routinely Tested
Western blot	Reported

Recommended Assay Procedure:

Caution: Our studies demonstrate that Mouse BD Fc Block™ anti-CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) and antibodies of rat IgG2b isotype may interfere with the reactivity of rmC5-3 antibody in a concentration-dependent manner. For inhibition of non-specific reactivity, we recommend use of purified mouse IgG at 10-100-fold excess. Other reported applications include western blot analysis. For in vitro and in vivo blocking of LPS binding to CD14, we recommend the NA/LE™ format of the alternate anti-mouse CD14 mAb 4C1/CD14 (Cat. No. 557896). Use of rmC5-3 antibody for immunohistochemical staining has been reported; however, we have been unable to reproduce those results at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554016	FITC Goat Anti-Rat Igs	0.5 mg	Polyclonal

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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References

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