BD Horizon™

Technical Data Sheet

BUV737 Rat Anti-Mouse CD14

Product Information

Material Number: 565309
Alternate Name: Cd14; CD14 antigen; Myeloid cell-specific leucine-rich glycoprotein
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: rmC5-3
Immunogen: Recombinant Mouse CD14
Isotype: Rat (LOU) IgG1, κ
Reactivity: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The rmC5-3 monoclonal antibody specifically binds to residues 308-322 of the hydrophilic region of mouse CD14. CD14 is a 53-55 kDa glycosphatidylinositol (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). CD14 serves as a receptor for LPS that can play a role in the cellular production of proinflammatory cytokines such as IL-1 and TNF. CD14 can be 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 naïve 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. 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 was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome of BD Horizon BUV395 with an Ex Max of 348-nm and an acceptor dye with an Em Max at 737-nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 filter. Due to the excitation of the acceptor dye by other laser lines, there may be significant spillover into channels detecting Alexa Fluor® 700-like dyes (e.g., 712/20-nm filter).

Due to spectral differences between labeled cells and beads, using BD™ CompBeads can result in incorrect spillover values when used with BD Horizon BUV737 reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different BUV737 reagents (e.g., CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.

Flow cytometric analysis of CD14 expression on mouse J774A.1 cells. Cells from the J774A.1 (Mouse macrophage, ATCC TIB-67) cell line were stained with either BD Horizon™ BUV737 Rat IgG1, κ Isotype Control (Cat. No. 564690; dashed line histogram) or BD Horizon BUV737 Rat Anti-Mouse CD14 antibody (Cat. No. 565309; solid line histogram). The fluorescence histogram showing CD14 expression (or Ig Isotype control staining) was derived from gated events with the forward and side light-scatter characteristics of viable J774A cells. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.
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™ BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon
BUV737 were removed.

Application Notes

Application

| Flow cytometry | Routinely Tested |

Suggested Companion Products

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<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<td>Stain Buffer (FBS)</td>
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<tr>
<td>564690</td>
<td>BUV737 Rat IgG1, κ Isotype Control</td>
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<td>563794</td>
<td>Brilliant Stain Buffer</td>
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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. 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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at
   www.bdbiosciences.com/colors.

References


Ferroero E, Hsieh CL, Francke U, Goyert SM. CD14 is a member of the family of leucine-rich proteins and is encoded by a gene syntenic with multiple receptor genes. J Immunol. 1990; 145(1):331-336. (Biology)


