BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer reduces nonspecific binding of cyanine-like dye conjugates to monocytes and other leukocytes

Abstract 2869

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Abstract

Nonspecific binding of monocytes to certain cyanine-like dye conjugated antibodies that are commonly used in flow cytometry for cell surface staining is well known. Although lesser in extent than monocytes, other leukocyte subsets can also exhibit nonspecific binding to cyanine-like dye conjugates. This poses a challenge for multicolor flow cytometric analysis utilizing these cyanine-like dye conjugates as dye-mediated nonspecific binding can mask the real expression.

Here we demonstrate that the BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer, a blocking buffer solution, eliminates nonspecific binding of cyanine-like dye conjugates to monocytes and other leukocyte populations. In this study we show that human whole blood stained with PE-Cy5, PE-Cy7, APC-Cy7, APC-H7, PE-CF594 and other tandem-dye conjugated antibodies in the presence of this new buffer exhibits the appropriate staining profile of these antibodies to monocytes and other leukocyte subsets.

We also observed that nonspecific binding of cyanine-like dye conjugated antibodies to myeloid and lymphoid subsets in mouse lymphoid tissues was diminished. BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer, while reducing nonspecific cyanine-like dye conjugates binding, did not impact the desirable antibody-mediated specific staining. Our data also show that cell viability is not impacted.

Materials and Methods

Materials:

1. MOPC-21 APC-Cy7 (BD Cat. No. 557873) 0.5 µg
2. MOPC-21 APC-H7 (BD Cat. No. 560167) 0.5 µg
3. MOPC-21 PE-Cy5 (BD Cat. No. 555750) 0.25 µg
4. MOPC-21 PE-Cy7 (BD Cat. No. 565573) 0.5 µg
5. X40 PE-CF594 (BD Cat. No. 562292) 0.5 µg
6. M5E2 PE-Cy7 (BD Cat. No. 557742) 1 µg
7. A95-1 PE-Cy7 (BD Cat. No. 552849) 1 µg
8. M1/70 PE-Cy7 (BD Cat. No. 552850) 0.25 µg
9. 2D7/CCR5 PE-Cy7 (BD Cat. No. 557752) 0.5 µg
10. ML5 PE-Cy7 (BD Cat. No. 561646) 1.0 µg
11. G155-178 PE-Cy7 (BD Cat. No. 552868) 0.5 µg
12. P67.6 PE-Cy7 (BD Cat. No. 333946) 0.125 µg
13. 10.1 PE-Cy7 (BD Cat. No. 561191) 1.0 µg
14. EH12.1 PE-Cy7 (BD Cat. No. 561272) 0.5 µg
15. UCHT1 PE-Cy7 (BD Cat. No. 563423) 0.25 µg
16. HIB19 PE-Cy7 (BD Cat. No. 560728) 0.25 µg
17. BD Pharmingen™ Stain Buffer (FBS) (BD Cat. No. 554656)
18. BD Pharmingen™ DAPI Solution (BD Cat. No. 564907)
19. BD Pharmingen™ 7-AAD (BD Cat. No. 559925)
20. BD Pharmingen™ Human BD Fc Block™ Reagent (BD Cat. No. 564220) 2.5 µg per test
21. BD Pharmingen™ Mouse BD Fc Block™ Reagent (BD Cat. No. 553141) 0.5 µg per test
22. BD Pharm Lyse™ Lysing Buffer (BD Cat. No. 555899)
23. BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer (BD Cat. No. 570002, 570003, 570004) 10 µl per test

Methods:

1. Fresh human leukocytes or PBMCs from healthy donors were suspended in 1% heat-inactivated FBS DPBS (BD Pharmingen™ Stain Buffer) after whole blood was treated with BD Pharm Lyse™ Lysing Buffer to remove erythrocytes or PBMCs were isolated using Ficoll-Paque™ PLUS (GE Healthcare) gradient centrifugation.
2. Fresh bone marrow and spleen cells were obtained from healthy C57BL/6 mice and were suspended in 1% heat-inactivated FBS DPBS (BD Pharmingen™ Stain Buffer).
3. Staining cells with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer: First mix BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer with antibody, then mix with target cell suspension. Incubate mixtures for 30 minutes at room temperature for human cells and at 4 °C for mouse tissue cells. After incubation, wash the cells twice with BD Pharmingen™ Stain Buffer (FBS).
4. To test potential effects of the BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer on the viability and light scattering profiles of human LWB, human PBMCs, mouse bone marrow cells and mouse splenocytes, mix cell suspensions with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer and incubate 30 minutes at room temperature for human LWB or PBMCs and at 4 °C for mouse tissue cells. Then wash twice with BD Pharmingen™ Stain Buffer (FBS). Add 7-AAD or DAPI solutions before flow cytometric analysis.
5. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ Software.

BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer eliminates nonspecific antibody binding on human blood and mouse bone marrow leukocytes

Figure 1A Human lysed whole blood cells

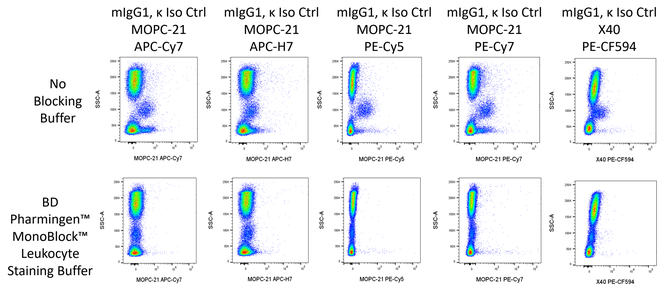

Figure 1B Human PBMCs

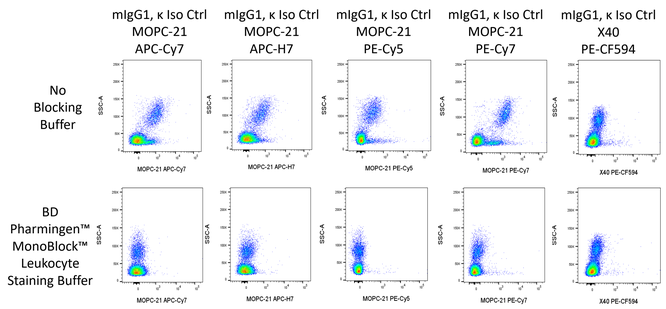

Figure 1: Human blood pre-treated with BD Pharm Lyse™ Lysing Buffer (1A) and human PBMCs (1B) were stained with APC-Cy7, APC-H7, PE-Cy5, PE-Cy7 and PE-CF594 conjugates of mouse IgG1 κ isotype controls with or without BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer.

Top rows: Background staining with cyanine-like dye conjugated antibodies on not just monocytes but also granulocytes (1A) and lymphocytes (1A and 1B).

Bottom rows: After addition of BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer, background staining was effectively reduced on all cell populations (1A and 1B).

Figure 2 Mouse bone marrow cells

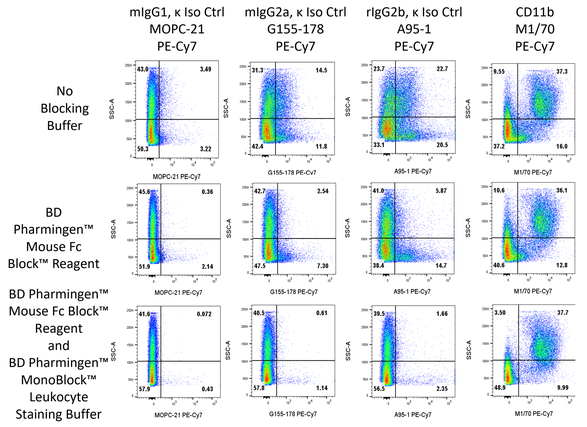

Figure 2: C57BL/6 bone marrow cells were stained with PE-Cy7 conjugates of mouse IgG1 κ isotype control (clone MOPC-21), mouse IgG2a κ isotype control (clone G155-178), rat IgG2b κ isotype control (clone A95-1), and mouse CD11b (clone M1/70, rIgG2b, κ) antibodies with or without BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer and BD Pharmingen™ Mouse Fc Block™ Reagent.

Top row: Background staining with PE-Cy7 conjugates on lymphoid and myeloid populations.

Middle row: Addition of BD Pharmingen™ Mouse Fc Block™ Reagent partially blocks the background staining dependent on the isotype of the antibody used.

Bottom row: Addition of BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer along with BD Pharmingen™ Mouse Fc Block™ Reagent effectively reduces background staining everywhere, while maintaining the CD11b antibody-specific staining on myeloid cells.

Figure 3 For mouse IgG2a κ isotype antibodies, BD Pharmingen™ Human Fc Block™ Reagent can further improve the blocking on human lysed whole blood cells

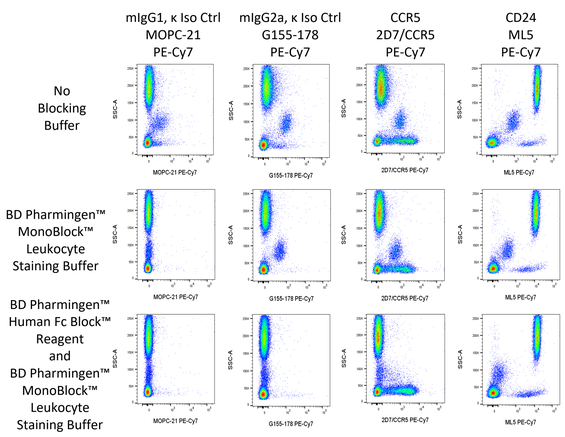

Figure 3: Human blood pre-treated with BD Pharm Lyse™ Lysing Buffer was stained with PE-Cy7 conjugates of mouse IgG1 κ isotype (clone MOPC-21) and the following mouse IgG2a κ isotype antibodies: mouse IgG2a κ isotype control (clone G155-178), anti-human CCR5 (clone 2D7/CCR5), and anti-human CD24 (Clone ML5), with or without BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer and BD Pharmingen™ Human Fc Block™ Reagent.

Top row: Background staining observed with PE-Cy7 conjugated antibodies.

Middle row: For mouse IgG2a κ isotype antibodies, in the absence of BD Pharmingen™ Human Fc Block™ Reagent only partial reduction of the background staining on monocytes is observed.

Bottom row: For mouse IgG2a κ isotype antibodies, addition of BD Pharmingen™ Human Fc Block™ Reagent can significantly reduce the background staining on monocytes.

BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer does not impact antibody-mediated specific binding, light scatter profiles or cell viability

Figure 4A Specific antibody staining on myeloid cells

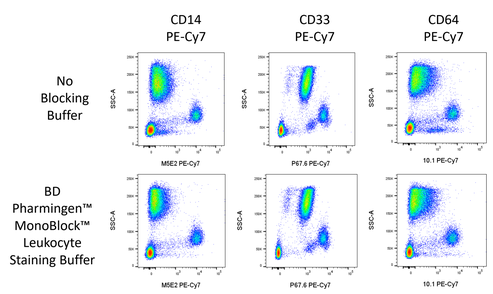

Figure 4A: Human blood pre-treated with BD Pharm Lyse™ Lysing Buffer was stained with PE-Cy7 conjugated anti-human CD14 (clone M5E2), CD33 (clone P67.6), and CD64 (clone 10.1) antibodies without (top row) or with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer (bottom row). CD14, CD33 and CD64 staining intensities on monocytes and/or granulocytes were not impacted by BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer, whereas the nonspecific background staining derived from PE-Cy7 conjugated antibodies on lymphocytes and/or granulocytes was reduced (bottom row).

Figure 4B Specific antibody staining on lymphocytes

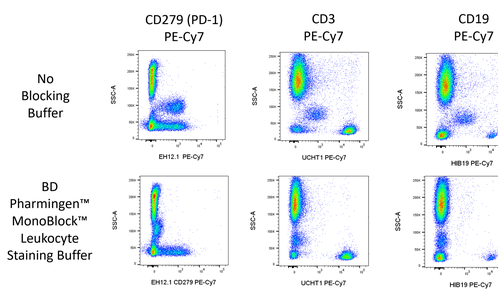

Figure 4B: Human blood pre-treated with BD Pharm Lyse™ Lysing Buffer was stained with PE-Cy7 anti-human CD279 (PD-1) (clone EH12.1), CD3 (clone UCHT1) and CD19 (clone HIB19) antibodies without (top row) or with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer (bottom row). CD279 (PD-1), CD3 and CD19 staining intensities on lymphocytes were not affected, whereas the nonspecific background staining derived from PE-Cy7 conjugate on monocytes was reduced (bottom row).

Figure 5A Light scatter profiles

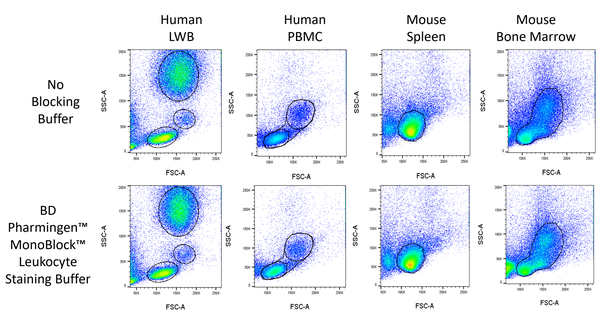

Figure 5A: Light scatter profiles of i) human LWB lymphocytes, monocytes and granulocytes, ii) human PBMC lymphocytes and monocytes, iii) mouse splenocytes and iv) mouse bone marrow lymphoid and myeloid cells are not impacted upon incubation with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer. Control cell populations (top row) have similar light scatter characteristics to those treated with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer (bottom row).

Figure 5B Cell viability

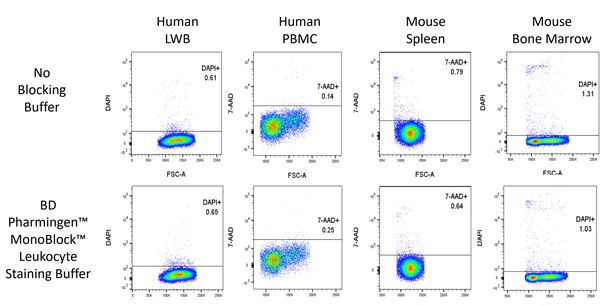

Figure 5B: The frequency of dead cells in i) human LWB lymphocytes, monocytes and granulocytes, ii) human PBMC, iii) mouse splenocytes and iv) mouse bone marrow was minimally changed after incubation with BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer for 30 minutes (bottom row) compared to control cell populations (top row).

Conclusions

1. BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer **can reduce cyanine-like dye conjugate background** on:
   - Human blood monocytes, lymphocytes and granulocytes
   - Mouse bone marrow lymphoid and myeloid cells
2. BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer **does not affect light scatter profiles and viability** of:
   - Human lysed whole blood cells
   - Human PBMCs
   - Mouse spleen and bone marrow cells
3. BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer **does not impact antibody-specific staining** of:
   - Human blood monocytes, lymphocytes and granulocytes
   - Mouse bone marrow myeloid cells
4. BD Pharmingen™ **Human Fc Block™ Reagent can further improve the blocking effect** of BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer for certain antibody isotypes.