True-Stain Monocyte Blocker™, an effective blocking buffer to eliminate non-specific cyanine-like dye-mediated monocyte binding

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Abstract

Some tandem fluorophores commonly used in flow cytometry have demonstrated a propensity to bind non-specifically to monocytes and macrophages when labeling is done on live cells. Although the effect is not limited to acceptor fluorophores from the cyanine dye family, they are the biggest culprit, and this non-specific binding can limit the ability to do multicolor flow cytometric analysis on lower density antigens. Here, we introduce True-Stain Monocyte Blocker™, which eliminates the nonspecific binding to monocytes and macrophages of dyes including PE/Cy7, PE/Cy5, PerCP/Cy5.5, APC/Cy7, APC/Fire™ 750, and PE/Dazzle™ 594. It does not affect antibody binding of monocyte antigens like CD64, CD14, and other tested markers. Multiple fluorophores can be blocked at once, and stability data shows no decrease in signal intensity compared to the conjugate alone. In addition, the reagent has no impact on cell viability.

Mock sorting experiments were performed with PBMCs to determine if the True-Stain Monocyte Blocker™ shows inhibitory or enhanced function on inflammatory cytokine/chemokine production. Although more data points are needed to ensure statistical relevance, our data indicates minimal to no effect above that of the conjugated antibody for IL-6, IL-8, IL-10 and MCP-1 production. We also tested True-Stain Monocyte Blocker™ for its ability to affect PBMC proliferation in response to anti-CD3/anti-CD28 stimulation. We observed minimal to no effect on proliferation by measuring BrdU incorporation.

Materials and Methods

- Blood was collected from healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll–Paque (GE Healthcare). After wash, cells were resuspended in RPMI-1640 with 10% FBS at the cell concentration of 1 X 10^6/ml.
- Mouse peritoneal cells were harvested from C57BL/6 mouse peritoneal cavity.
- Cell stimulation: PBMCs were stimulated with either 5 µg/mL of Con-A (Sigma C5275) or with 2 µg/mL of LEAF™ anti-human CD3 (UCHT1) (Cat. No. 300414) and 2 µg/mL of LEAF™ anti-human CD28 (CD28.2) (Cat. No. 302914) for 3 days.

Reagents used:

- True-Stain Monocyte Blocker™ (Cat. No. 426102)
- Mouse IgG_1 isotype control (clone MOPC-21)
- MOPC-21 PE/Cy7(Cat. No. 400126)
- MOPC-21 PerCP/Cy5.5 (Cat. No. 400150)
- MOPC-21 APC/Cy7 (Cat. No. 400128)
- MOPC-21 PE/Dazzle™ 594 (Cat. No. 400176)
- Anti-human CD14 PE/Cy7 (Cat. No. 325618)
- Anti-human CD14 PerCP/Cy5.5 (Cat. No. 325622)
- Anti-human CD14 APC/Cy7 (Cat. No. 325620)
- Anti-human CD14 PE/Dazzle™ 594 (Cat. No. 325634)
- Anti-human CD64 (FcγRI, and FcR I) PE/Cy7 (Cat. No. 305022)
- Anti-human CD3 PE/Cy7 (Cat. No. 300420)
- Anti-human CD16 APC/Cy7 (Cat. No. 302018)
- Anti-human CD8 APC (Cat. No. 344722)
- Anti-human CD4 FITC (Cat. No. 300506).
- Staining was done by mixing antibody with or without True-Stain Monocyte Blocker™ to 100 µL of human whole blood (or 1 X 10^6 cells in 100 µL volume) for 15 minutes. RBC were lysed with RBC Lysis buffer (420301) followed by washing 1X with cell staining buffer.
- BrdU incorporation was detected by using the Phase-Flow™ FITC BrdU kit (Cat. No. 370304)
- Cytokine levels were detected using the LEGENDplex™ Human inflammation panel (13 plex) (Cat. No. 740118)
- Flow cytometry data was collected using either a BD LSRFortessa™, BD FACSCanto™ II, BD™ LSRII. Data was analyzed using FlowJo® software.

Conclusions

True-Stain Monocyte Blocker™ can be used to eliminate unwanted monocyte binding to cyanine and related dyes for flow cytometric analysis.

Figure 1. True-Stain Monocyte Blocker™ blocks fluorophore-related dye binding to monocytes

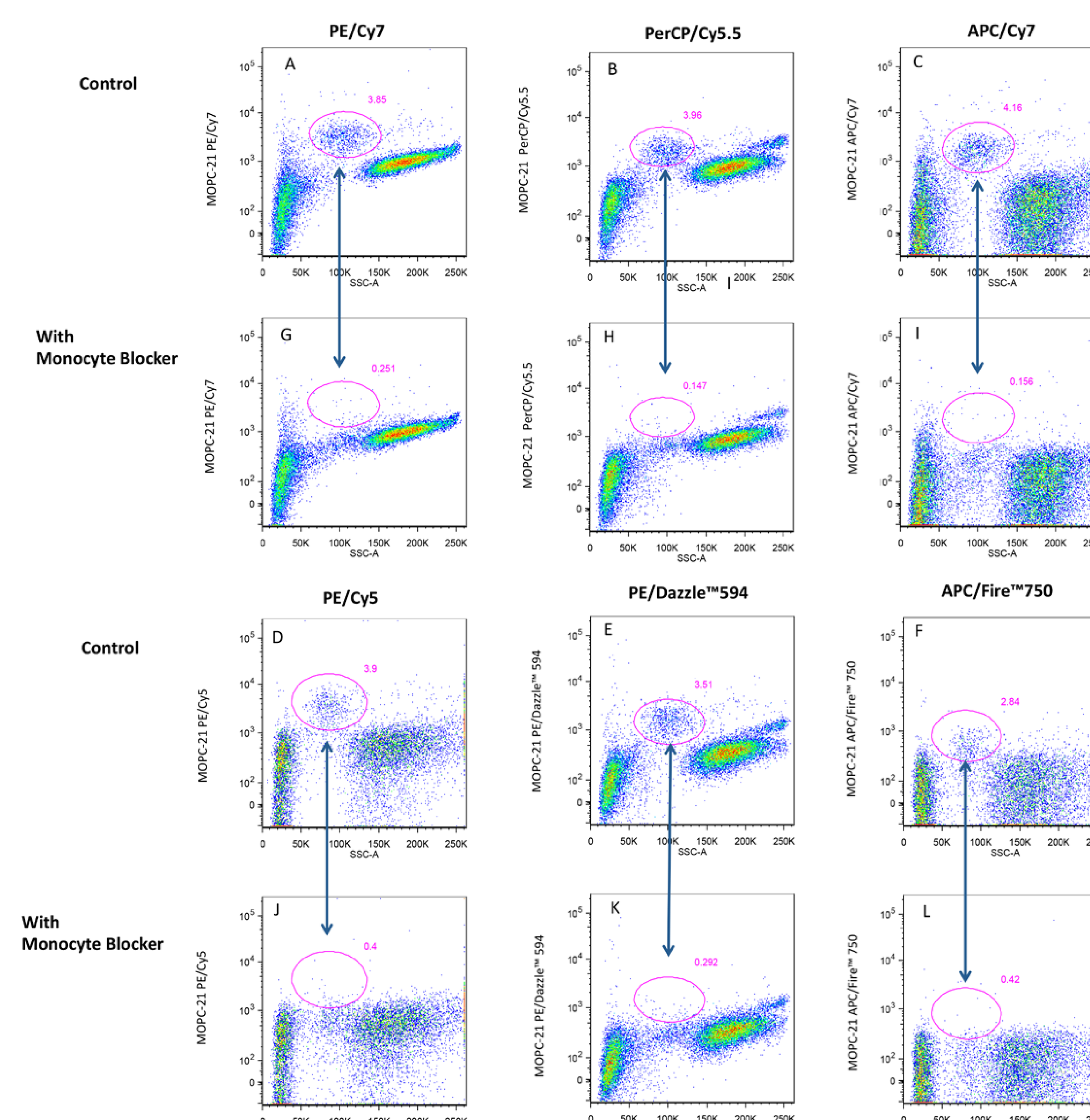

Figure 1. Human blood was stained with isotype control (MOPC-21) conjugates of PE/Cy7 (A), PerCP/Cy5.5 (B), APC/Cy7 (C), PE/Cy5 (D), PE/Dazzle™ 594 (E), and APC/Fire™ 750 (F). Monocytes show staining with all of these cyanine dyes (ovals, A-F). The monocyte blocker efficiently eliminates this binding, as shown by the lack of events in the ovals in plots G through L.

Figure 2. True-Stain Monocyte Blocker™ blocks staining on murine cells

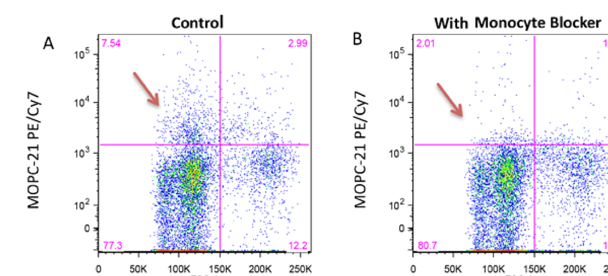

Figure 2. Mouse resident peritoneal cells were stained with MOPC-21 PE/Cy7 panels A and B. Monocytes/macrophages show binding to PE/Cy7 as shown in plot A (identified by red arrows). This staining is eliminated when using blocking reagent as seen in panel B.

Figure 3. True-Stain Monocyte Blocker™ does not interfere with antibody-specific staining

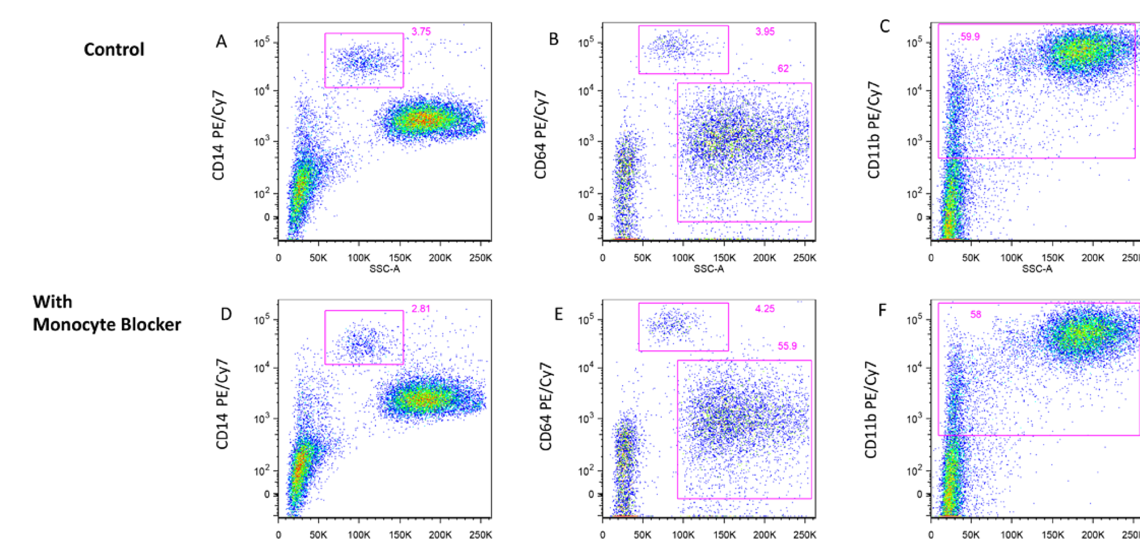

Figure 3. Human blood was stained without (panels A-C) or in the presence of monocyte blocker (panels D-F) for monocyte- and granulocyte- specific CD markers CD14 PE/Cy7, CD64 PE/Cy7 and CD11b PE/Cy7. Monocyte and granulocyte populations stain equivalently regardless of the presence of the blocker. Small percentage differences may be seen between treatment groups which may reflect statistical variability.

Figure 4. True-Stain Monocyte Blocker™ does not interfere with tandem dye cocktail staining

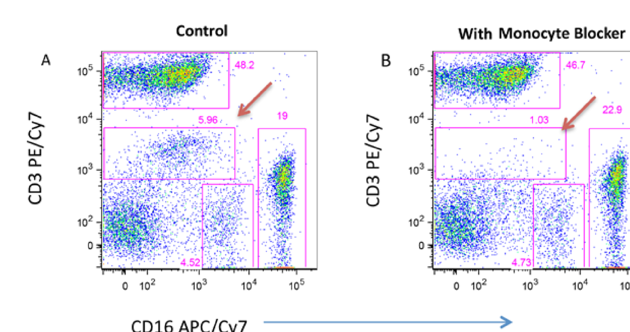

Figure 4. Human blood was stained with CD3 PE/Cy7 and CD16 APC/Cy7 without (A) or with monocyte blocker (B). Monocyte staining with the cyanine dyes is observed (by gated events identified by the red arrows) in (A). The monocyte blocker eliminates monocyte staining by cyanine dyes, but maintains the expected CD3/CD16 2-color staining profile (B). The same compensation matrix was used for both samples.

Figure 5. Unwanted monocyte binding is a viable cell phenomenon

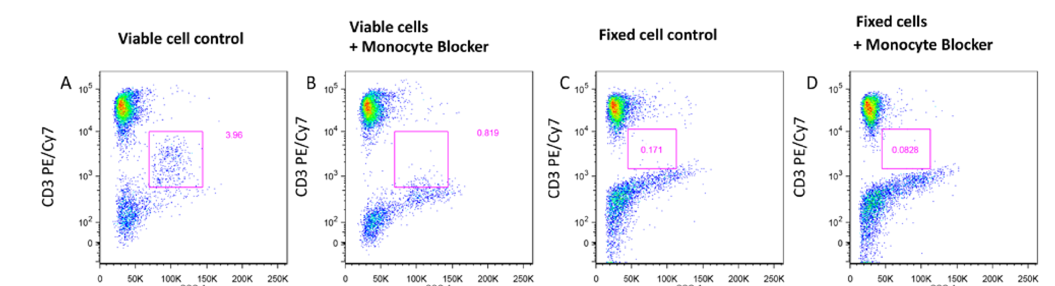

Figure 5. Viable monocytes bind cyanine dyes (A); fixed monocytes do not (C). Human PBMCs were stained with CD3 PE/Cy7 prior to fixation (A, B) or after fixation (C, D). Monocyte blocker eliminates the unwanted dye binding to monocytes in viable cells, but is not needed with fixed cell staining.

Figure 6. True-Stain Monocyte Blocker™ does not affect T cell proliferation

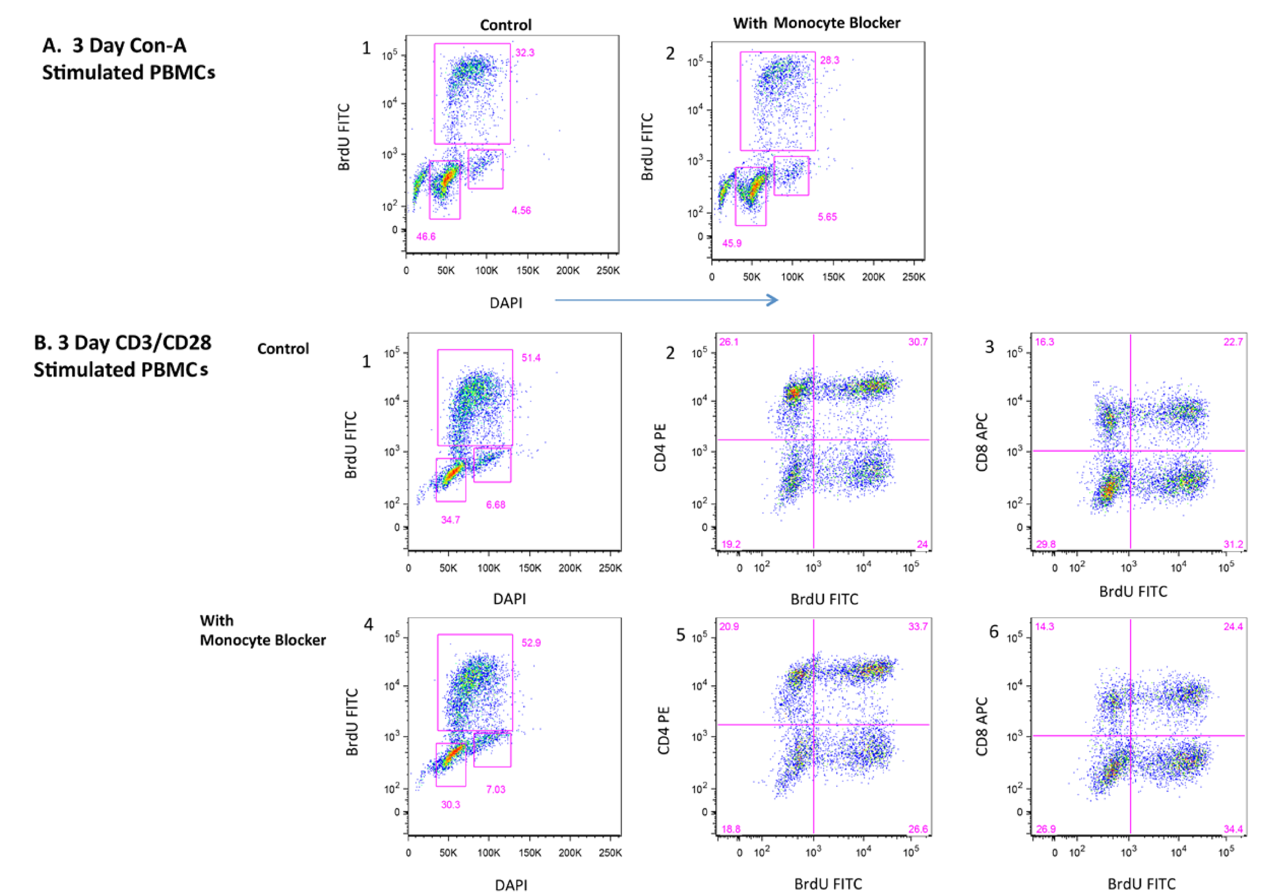

Figure 6A: Human PBMCss were treated with monocyte blocker in the presence of 5µg/mL of Con-A for 3 days. Cells were loaded with BrdU for 1 hour then stained using Phase-Flow™ FITC BrdU kit. Equivalent BrdU incorporation was observed with the monocyte blocker treatment (A2) compared to the control (A1) .

Figure 6B: Human PBMCs were stimulated for 3 days with anti-CD3/CD28 antibodies with (B 4-6) or without monocyte blocker (B 1-3). Cells were loaded for 1 hour with BrdU then stained with anti-BrdU FITC, CD4 PE, and CD8 APC using the Phase-Flow™ FITC BrdU kit. Equivalent BrdU incorporation levels were observed with or without monocyte blocker. CD4 and CD8 populations showed equivalent levels of BrdU incorporation compared to the control group.

Figure 7. Cytokine production proceeds similarly in the presence of True-Stain Monocyte Blocker™

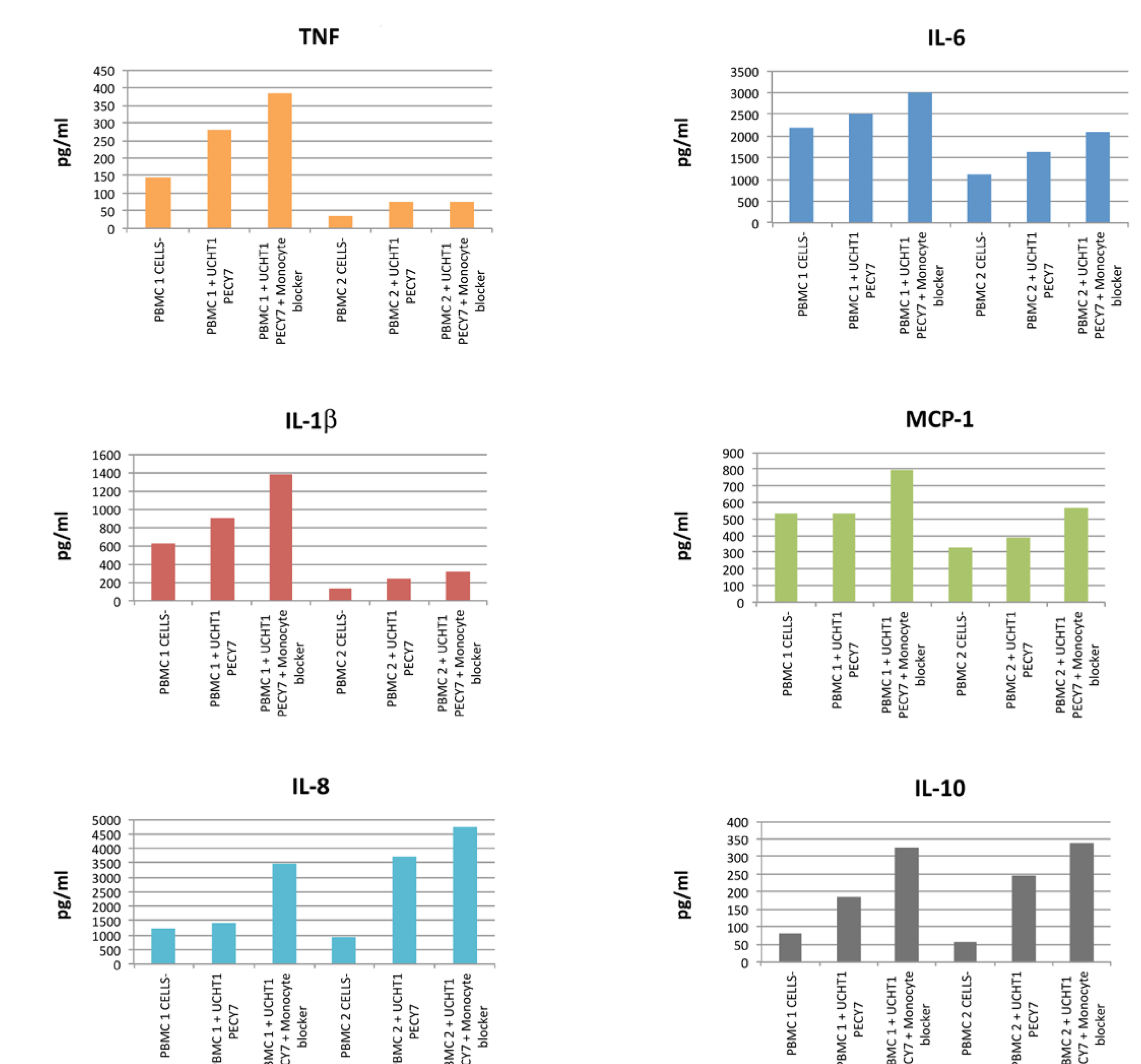

Figure 7. PBMCs were isolated from two donors. Cells were divided into 3 groups, Cells, UCHT1 PE/Cy7 (optimal concentration), or UCHT1 PE/Cy7 (optimal concentration) + monocyte blocker. Cells were stained for 15 minutes then washed and plated in complete RPMI-1640. Supernatants were harvested the next day and assayed using the LEGENDplex™ human inflammation panel (13 plex). Of the 13 analytes tested, only 6 showed detectable levels. Both of the donors produced small cytokine responses with either the CD3 PE/Cy7 alone or the CD3 PE/Cy7 plus monocyte blocker. Determining whether this response is significant would require additional donors and replicates.

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