Comprehensive Analysis of Buffers Used to Reduce False Positives for Intracellular Flow Cytometry

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INTRODUCTION

Nonspecific binding between antibody dye conjugates and live human monocytes and live macrophages is a well-known phenomenon. Commercially available reagents are frequently used to block interactions between antibodies or dyes and the surface of live primary cells. However, limited blocking studies have been performed on paraformaldehyde fixed and methanol permeabilized human peripheral blood mononuclear cells (PBMCs). Fixation and permeabilization are required for antibody-based analysis of cell signaling pathways and transcription factors using intracellular flow cytometry. Here, we aim to determine if the sample preparation method impacts staining specificity and evaluate reagents to reduce false positive results.

METHODS

Human PBMCs were paraformaldehyde (4%) fixed and methanol (90%) permeabilized, then stained with a rabbit IgG isotype control antibody conjugated to a variety of fluorescent dyes, including Alexa Fluor®, mFluor™, Pacific Blue™, PE, PE-Cy®7, APC, and APC-Cy®7 and analyzed by flow cytometry. For small molecule dyes, different molar dye to IgG ratios (degree of labeling, DOL) were also included in the analysis. A series of blocking buffers were compared for their ability to block nonspecific interactions between antibody dye conjugates and fixed/permeabilized cells. Monoclonal antibody conjugates targeting phospho-proteins and transcription factors were tested to ensure that the blocking buffers did not alter specific staining.

CONCLUSIONS

Nonspecific staining was observed in unblocked cells and varied by dye and DOL. Addition of blocking buffers reduced nonspecific dye-related background in fixed/permeabilized PBMCs. Blocking buffers had minimal effect on target-specific intracellular antibody staining. In conclusion, blocking buffers successfully reduced dye-conjugated antibody background staining in fixed/permeabilized human PBMCs. These reagents provide an easy-to-use method to reduce possible false positive signal in intracellular flow cytometry experiments.

FlouroClear Blocking Buffer Reduces Dye-Specific Background

Figure 1A

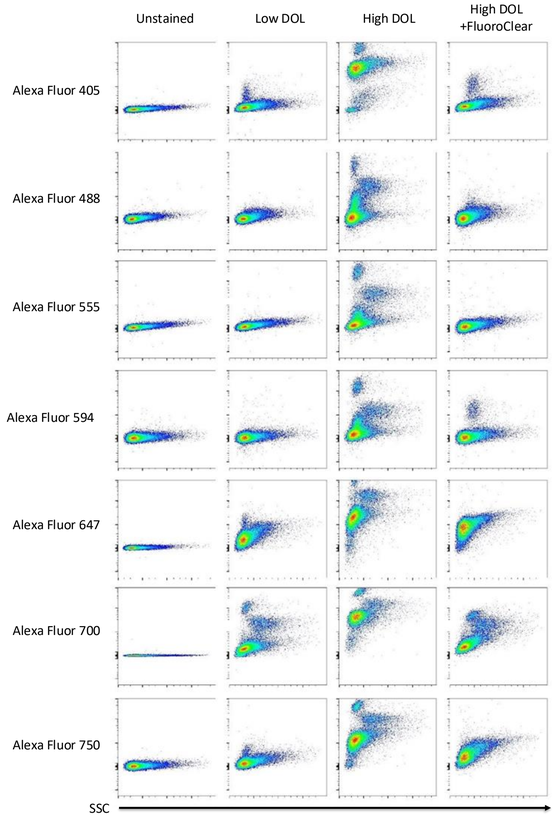

Figure 1B

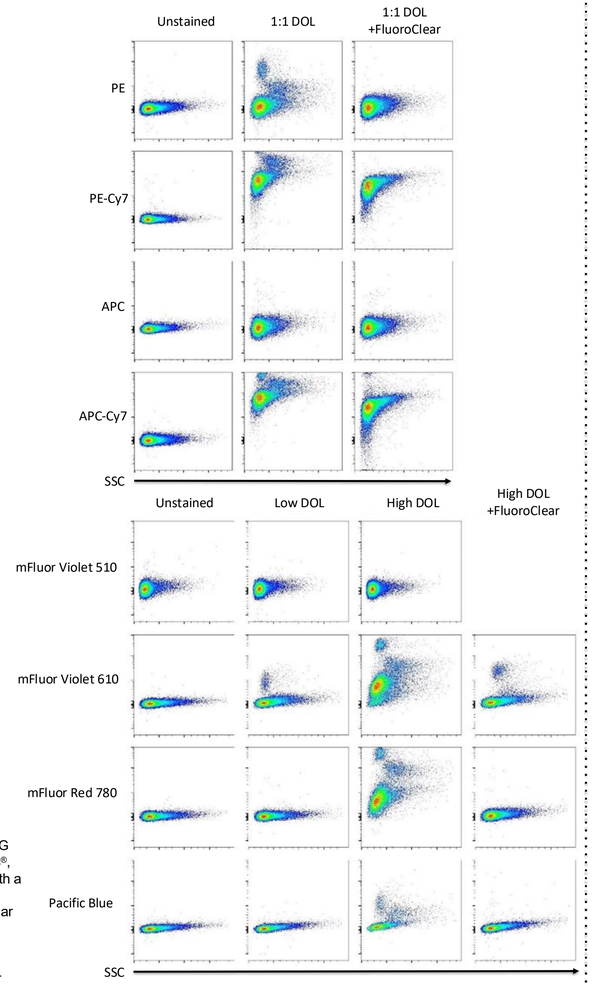

Figure 1A, 1B
Flow cytometric analysis of fixed/permeabilized PBMCs unstained or stained with rabbit IgG isotype control antibody conjugated to a variety of fluorescent dyes, including Alexa Fluors®, mFluors™, Pacific Blue™, PE, PE-Cy®7, APC, and APC-Cy®7. Staining was performed with a 1:1 DOL for protein dyes, and low (< 5) and high DOLs (> 5) for small molecule dyes. Additionally, high DOL conjugates and protein dye conjugates were tested with FluoroClear Blocking Buffer #33449.

Table 1
Analysis of the percentages of lymphocytes or monocytes with false positive signal greater than unstained control in the high DOL conjugates and protein dye conjugates.

	Alexa 405	Alexa 488	Alexa 555	Alexa 594	Alexa 647	Alexa 700	Alexa 750	mFluor v510	mFluor v610	mFluor r780	Pacific Blue	PE	PE-Cy7	APC	APC-Cy7
Lymphocytes %	96	20	21	21	93	98	98	0	84	75	2	16	94	8	89
Monocytes %	88	26	31	30	43	45	45	1	41	43	22	27	32	17	32

Antibody-Specific Staining Retained with FluoroClear

Figure 2

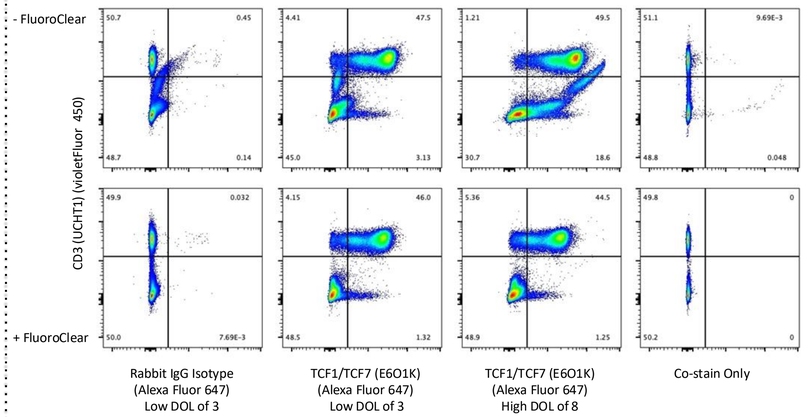

Figure 2
Flow cytometric analysis of fixed/permeabilized PBMCs stained with Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) #2985 or TCF1/TCF7 (E6O1K) Rabbit mAb (Alexa Fluor® 647 Conjugate) #83268 with varying DOLs as indicated, co-stained with CD3 (UCHT1) Mouse mAb (violetFluor 450 Conjugate) #61347. Staining was performed without blocking buffer (top row) or with FluoroClear Blocking Buffer #33449 (bottom row).

Figure 3

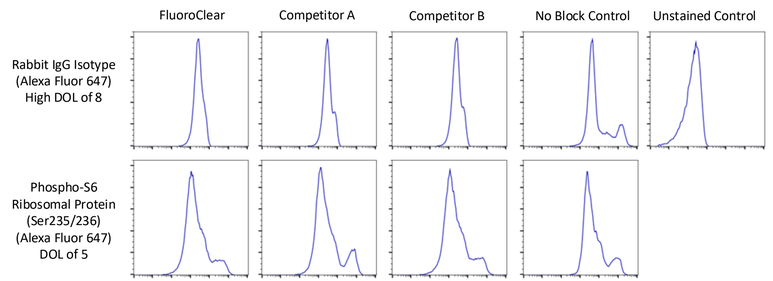

Figure 3
Flow cytometric analysis of the effects of FluoroClear Blocking Buffer #33449 compared to competitor blocking reagents on fixed/permeabilized PBMCs stained with Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) #2985 (top row) or Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) #4851 (bottom row), compared to unstained and no blocking buffer controls.

FluoroClear Blocking Buffer #33449
Available at Cell Signaling Technology

Cell Signaling TECHNOLOGY®

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