CellBlox™ Plus Blocking Buffer

Catalog Numbers C001T02F01, C001T03F01, and C001T06F01

Pub. No. MAN0029764 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ CellBlox™ Plus Blocking Buffer is formulated to block nonspecific binding of Invitrogen™ NovaFluor™ labels with cells. These nonspecific interactions can result in higher background labeling. CellBlox™ Plus Blocking Buffer is a non-antibody blocking solution, and must be used every time a NovaFluor™ dye is used for labeling any cell type to minimize background labeling (Figure 1).

CellBlox™ Plus Blocking Buffer is also recommended for use with cyanine-based dyes or tandem dyes to block nonspecific interactions with monocytes, macrophages, and other cell types to minimize background labeling (Figure 2).

Use of CellBlox™ Plus Blocking Buffer requires minimal change to most flow cytometry staining protocols. Before labeling cells, add 5 µL of CellBlox™ Plus Blocking Buffer to antibody mixture being used to label stained samples in a final staining volume of 100 µL.

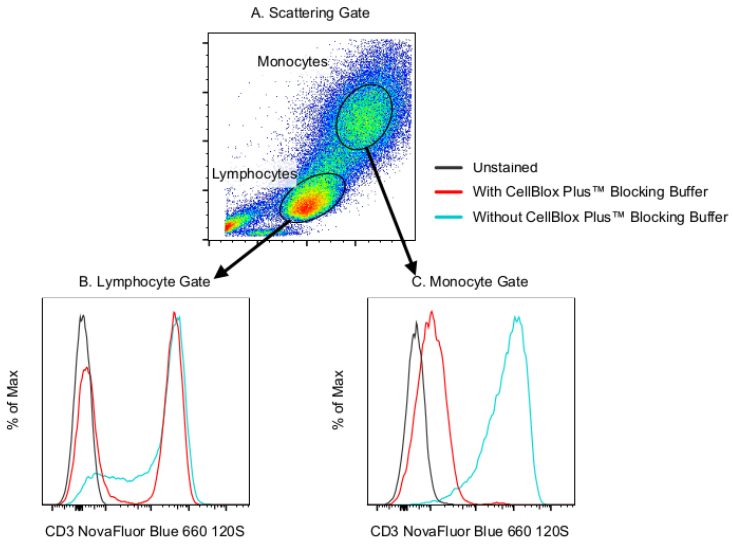

Figure 1 CellBlox™ Plus Blocking Buffer reduces nonspecific interactions of NovaFluor™ dyes when using cells.

CD3 labeling combined with CellBlox™ Plus Blocking Buffer is shown to reduce background in lymphocytes and nonspecific labeling of monocytes and macrophages. Peripheral blood mononuclear cells (PBMCs) were left unstained (black) and stained with (red) or without (blue) CellBlox™ Plus Blocking Buffer and CD3 monoclonal antibody NovaFluor™ Blue 660-120S conjugate (Cat. No. H002T03B08). A forward scatter vs. side scatter pseudocolor plot shows (A) lymphocyte and monocyte gates. Histogram overlay plots show the expression of CD3 within the (B) lymphocyte and (C) monocyte gates. Data were acquired in the B7 channel on a 5-laser Cytek™ Aurora flow cytometer.

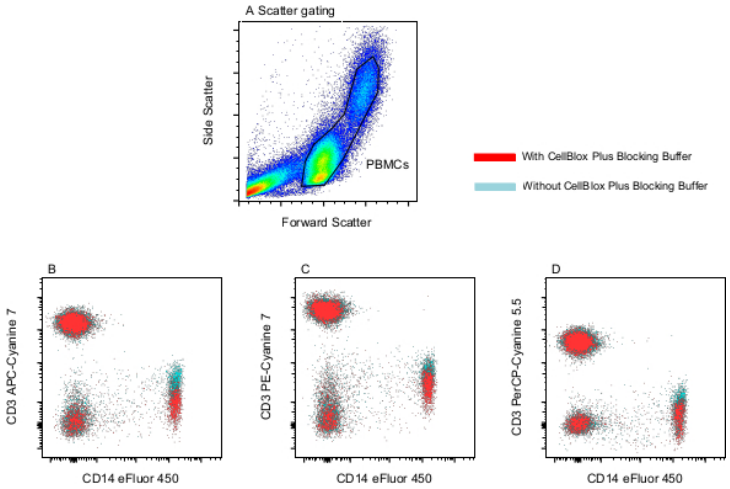

Figure 2 CellBlox™ Plus Blocking Buffer reduces nonspecific interactions of cyanine tandem dyes when using cells.
Peripheral blood mononuclear cells (PBMCs) were labeled with a CD14 direct conjugate of eFluor™ 450 and a CD3 direct conjugate of either APC-Cyanine 7, PE-Cyanine 7, or PerCP-Cyanine 5.5, with and without the addition of CellBlox™ Plus Blocking Buffer (Cat. No. C001T03F01). (A) A forward scatter vs. side scatter density plot shows a combined lymphocyte and monocyte gate. A dot plot overlay of CD3 and CD14 expression displays cell labeling with (red) and without (blue) CellBlox™ Plus Blocking Buffer. Use of CellBlox™ Plus Blocking Buffer is shown to reduce nonspecific interactions with monocytes and minimize background labeling of cells with (B) PE-Cyanine 7, (C) APC-Cyanine 7, and (D) PerCP-Cyanine 5.5 tandem dyes. Data were acquired on a 5-laser Cytek™ Aurora flow cytometer using the B9 detector for PerCP-Cyanine 5.5, the YG9 detector for PE-Cyanine 7, and the R7 detector for APC-Cyanine 7.

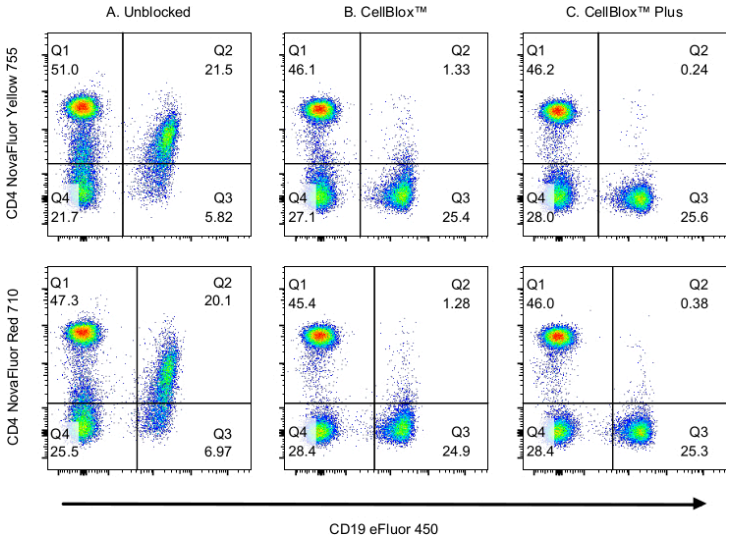

Figure 3 CellBlox™ Plus Blocking Buffer reduces nonspecific interactions when compared to not using a blocking buffer or using CellBlox™ Blocking Buffer.

The data demonstrate the ability of CellBlox™ Plus Blocking Buffer to improve blocking compared to standard CellBlox™ Blocking Buffer. Peripheral blood mononuclear cells (PBMCs) were (A) unblocked, (B) blocked with CellBlox™ Blocking Buffer (Cat. No. B001T06F01), or (C) blocked with CellBlox™ Plus Blocking Buffer (Cat. No. C001T06F01). Cells were then stained with (top) CD4 monoclonal antibody NovaFluor™ Yellow 755, or (bottom) CD4 monoclonal antibody NovaFluor™ Red 710. All cells were co-stained with CD19 monoclonal antibody eFluor™ 450 (48-0199-42). Two-dimensional pseudocolor plots of CD4 vs. CD19 show that the CellBlox™ Plus Blocking Buffer reduces nonspecific binding compared to staining with CellBlox™ Blocking Buffer. Data were acquired on a 5-laser Cytek™ Aurora flow cytometer.

Contents and storage

Cat. No.	Amount	Storage
C001T02F01	25 tests	4°C (Do not freeze)
C001T03F01	100 tests	
C001T06F01	500 tests	

Required materials not provided

- eBioscience™ Flow Cytometry Staining Buffer (Cat. No. 00-4222-26)
- Primary conjugated antibodies
- 12 × 75 mm round-bottom polystyrene test tubes or U- or V-bottom polystyrene microplates

Add CellBlox™ Plus Blocking Buffer to an antibody mixture

1. Prepare a single cell suspension as described in **BestProtocols: Cell Preparation for Flow Cytometry Protocols**.
2. Aliquot the cell suspension containing 10^3–10^8 cells to each sample tube or well.

   Note: If blocking Fc-mediated interactions is desired, Fc block can be added at this step before use of CellBlox™ Plus Blocking Buffer.
3. Prepare an antibody mixture of conjugated antibodies at predetermined optimal concentrations of each antibody conjugate. Mix well after the addition of each antibody.

   Note: If more than one Super Bright, Brilliant Ultra Violet™, or Brilliant Violet™ dye is included in the antibody mixture, then we recommend including one of these buffers to block dye-dye interactions: Super Bright Complete Staining Buffer at 5 µL per sample, Brilliant Stain Buffer at 50 µL per sample, or Brilliant Stain Buffer Plus at 10 µL per sample.
4. Add 5 µL of CellBlox™ Plus Blocking Buffer for every sample to be labeled directly into the antibody mixture to a final staining volume of 100 µL per sample.

   Note: Use only 5 µL of CellBlox™ Plus Blocking Buffer regardless of how many NovaFluor-conjugated antibodies are in the antibody cocktail.

   Note: When preparing antibody mixtures, it is recommended that the total volume be more than needed to ensure sufficient solution when pipetting. For example, if the total volume is 400 µL then make 440 µL that contains 22 µL of CB+.
5. Add a volume of antibody mixture containing CellBlox™ Plus Blocking Buffer to aliquoted cell samples with 100 µL as a final staining volume per sample.
6. Incubate samples for 30 minutes at 2–8°C, protected from light.
7. Wash the cells by adding 2 mL eBioscience™ Flow Cytometry Staining Buffer per sample. Centrifuge at 400–600 × *g* for 5 minutes. Discard supernatant.
8. Repeat step 7.
9. Resuspend cells in an appropriate volume of eBioscience™ Flow Cytometry Staining Buffer.
10. Analyze samples by flow cytometry.

Optimize the experiments

- Always use CellBlox™ Plus Blocking Buffer with NovaFluor™ dyes when labeling cells for optimal background reduction.
- CellBlox™ Plus Blocking Buffer is compatible with all fluorophores and Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stains.
- CellBlox™ Plus Blocking Buffer can be used with any fluorophore-antibody conjugate as a high-performance monocyte and macrophage blocking solution.
- CellBlox™ Plus Blocking Buffer is compatible with other blocking reagents, such as Fc Block, blocking proteins, Brilliant Stain Buffer, and Super Bright Complete Staining Buffer.
- CellBlox™ Plus Blocking Buffer is not needed when labeling antibody-capture beads.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029764 A.0

Revision	Date	Description
A.0	17 July 2023	New document for CellBlox™ Plus Blocking Buffer.