CellBlox™ Blocking Buffer

Catalog Numbers B001T02F01, B001T03F01, and B001T06F01

Pub. No. MAN0026225 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™ Blocking Buffer is formulated to block nonspecific binding of Invitrogen™ NovaFluor™ labels with cells. These nonspecific interactions can result in higher background labeling. CellBlox™ Blocking Buffer is a non-antibody, non-protein–based blocking solution, and should be used every time a NovaFluor™ dye is used for labeling any cell type to minimize background labeling (Figure 1).

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

Use of CellBlox™ Blocking Buffer requires minimal change to most flow cytometry staining protocols. Add 5 µL of CellBlox™ Blocking Buffer directly to a cell suspension containing 10^3–10^8 cells prior to the addition of an antibody, with 100 µL as a final staining volume. CellBlox™ Blocking Buffer may instead be added to an antibody mixture prior to labeling cells, by adding 5µL CellBlox™ Blocking Buffer for every stained sample to be labeled with the antibody mixture, with 100 µL as a final staining volume.

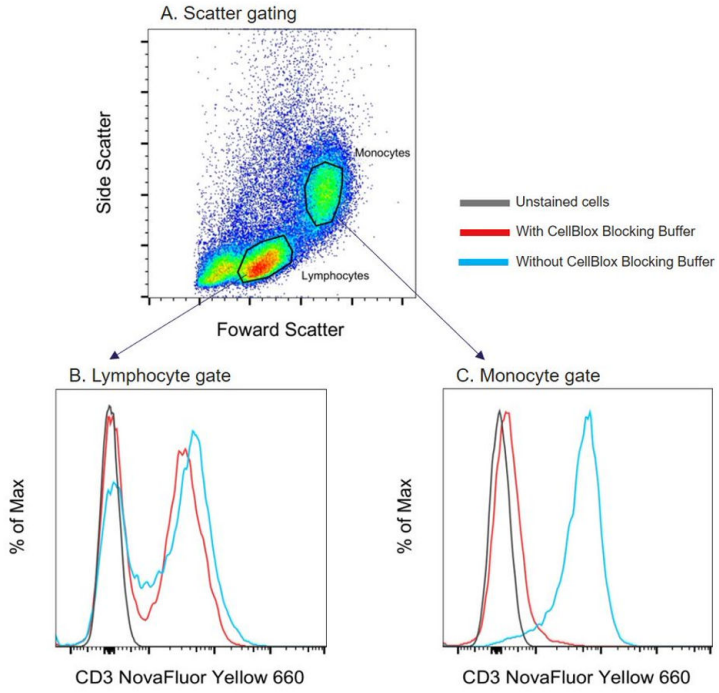

Figure 1 CellBlox™ Blocking Buffer mitigates nonspecific interactions of NovaFluor™ dyes when using cells.

Peripheral blood mononuclear cells (PBMCs) were either unlabeled (grey) or labeled with CD3 Monoclonal Antibody (clone UCHT1), NovaFluor™ Yellow 660 conjugate (Cat. No. H002T03Y04) with (red) and without (blue) the addition of CellBlox™ Blocking Buffer (Cat. No. B001T06F01). (A) Forward scatter vs. side scatter density plot shows lymphocyte and monocyte gating. Histogram overlay plots of CD3 expression are shown using a (B) lymphocyte gate and a (C) monocyte gate. CD3 labeling combined with CellBlox™ Blocking Buffer is shown to reduce background in lymphocytes and reduce nonspecific labeling of monocytes and macrophages, as compared with CD3 labeling without CellBlox™ Blocking Buffer, leading to an improvement in resolution. Data were acquired on a 4-laser Invitrogen™ Attune™ NxT Flow Cytometer using the 561-nm laser with a 695/40-nm bandpass filter.

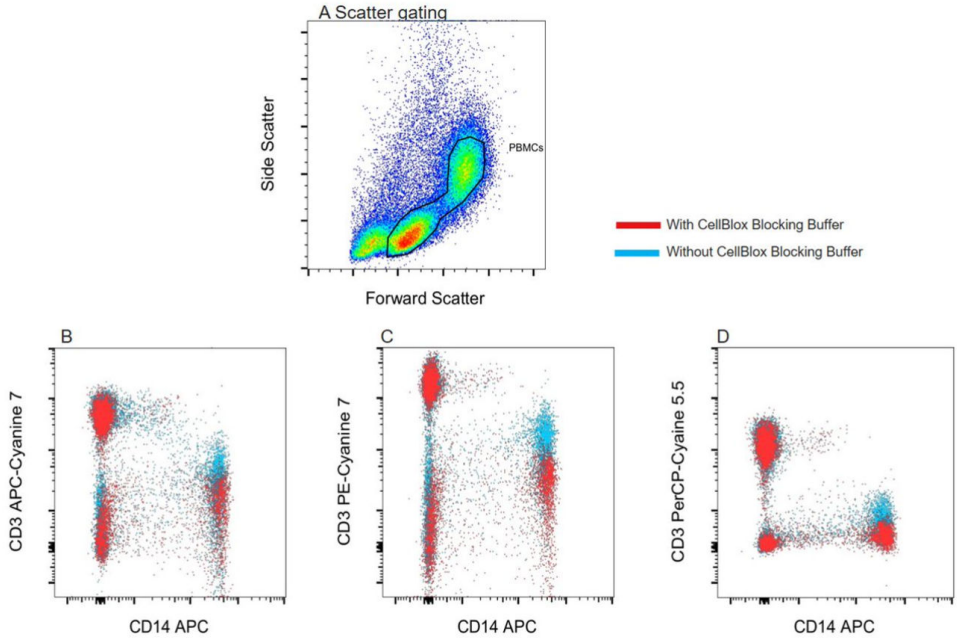

Figure 2 CellBlox™ Blocking Buffer mitigates nonspecific interactions of cyanine tandem dyes when using cells.

Peripheral blood mononuclear cells (PBMCs) were labeled with a CD14 direct conjugate of APC and a CD3 direct conjugate of APC-Cyanine 7, PE-Cyanine 7, and PerCP-Cyanine 5.5, with and without the addition of CellBlox™ Blocking Buffer (Cat. No. B001T06F01). (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™ Blocking Buffer. Use of CellBlox™ 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 4-laser Invitrogen™ Attune™ NxT Flow Cytometer using a 488-nm laser with a 695/40-nm bandpass for PerCP-Cyanine 5.5, a 561-nm laser with a 780/60-nm bandpass filter for PE-Cyanine 7, and a 638-nm laser with a 780/60-nm bandpass filter for APC-Cyanine 7.

Contents and storage

Cat. No.	Amount	Storage
B001T02F01	25 tests	4°C (Do not freeze)
B001T03F01	100 tests	
B001T06F01	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™ Blocking Buffer to an antibody mixture (preferred protocol)

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.
3. Prepare an antibody mixture of conjugated antibodies at predetermined optimal concentrations of each antibody conjugate. Mix well after the addition of each antibody.
4. Add 5 µL of CellBlox™ Blocking Buffer for every sample to be labeled directly into the antibody mixture to a final staining volume of 100 µL per sample. For example, if preparing enough antibody mixture for use with 10 samples, add 50 µL of CellBlox™ Blocking Buffer to the antibody mixture.
5. Add a volume of antibody mixture containing CellBlox™ 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 or, if staining for intracellular targets, proceed to **BestProtocols: Staining Intracellular Antigens for Flow Cytometry**.

Add CellBlox™ Blocking Buffer to bulk cell samples

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 for all samples to be labeled.
3. Add 5 µL of CellBlox™ Blocking Buffer to the bulk cell suspension for every sample to be labeled. For example, if preparing enough bulk cells for use with 10 samples, add 50 µL of CellBlox™ Blocking Buffer to the bulk cell suspension.
4. Add the cell suspension containing CellBlox™ Blocking Buffer to each sample tube or well.
5. Add an appropriate amount of each antibody conjugate to each cell suspension aliquot containing CellBlox™ Blocking Buffer 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 of 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 or, if staining for intracellular targets, proceed to **BestProtocols: Staining Intracellular Antigens for Flow Cytometry**.

Add CellBlox™ Blocking Buffer to individual cell samples

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.
3. Add 5 µL of CellBlox™ Blocking Buffer directly to each sample prior to staining cells.
4. Add an appropriate amount of each antibody conjugate to each cell suspension aliquot containing CellBlox™ Blocking Buffer with 100 µL as a final staining volume per sample.
5. Incubate samples for 30 minutes at 2–8°C, protected from light.
6. Wash the cells by adding 2 mL of eBioscience™ Flow Cytometry Staining Buffer per sample. Centrifuge at 400–600 × *g* for 5 minutes. Discard supernatant.
7. Repeat step 6.
8. Resuspend cells in an appropriate volume of eBioscience™ Flow Cytometry Staining Buffer.
9. Analyze samples by flow cytometry or, if staining for intracellular targets, proceed to **BestProtocols: Staining Intracellular Antigens for Flow Cytometry**

Optimize your experiments

- Always use CellBlox™ Blocking Buffer with NovaFluor™ dyes when labeling cells for optimal background reduction.
- CellBlox™ Blocking Buffer is compatible with all fluorophores and Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stains.
- CellBlox™ Blocking Buffer can be used with any fluorophore-antibody conjugate as a high-performance monocyte and macrophage blocking solution.
- CellBlox™ Blocking Buffer is compatible with other blocking reagents, such as Fc Block, blocking proteins, Brilliant Stain Buffer, and Super Bright Complete Staining Buffer.
- CellBlox™ Blocking Buffer is not required 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. MAN0026225

Revision	Date	Description
A.0	04 February 2022	New user guide for CellBlox™ Blocking Buffer.