CountBright™ Plus Absolute Counting Beads and CountBright™ Plus Ready Tubes

Catalog Numbers C36950, C36995, and C40000

Pub. No. MAN0018850 Rev. B.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

Flow cytometry provides a rapid method to quantify cell characteristics, however most flow cytometers cannot directly provide the concentration or absolute count of cells in a sample. Absolute cell counts have been widely used in quantifying cell populations and disease progression research, including studies of stem cells, HIV/AIDS, and leukemia.

CountBright™ Absolute Counting Beads and CountBright™ Plus Absolute Counting Beads are microparticles that can be used with most cell types, including no-wash/lysed whole blood. The microspheres are compatible with UV (350 nm), violet (405 nm), blue (488 nm), yellow (532 nm), green (561 nm), red (633 nm), and NIR (810 nm, CountBright™ Plus only) excitation sources and emit fluorescence ranging from 385 nm to 860 nm.

CountBright™ Plus Ready Tubes provide all the advantages of CountBright™ Plus Absolute Counting Beads in a convenient, ready-to-use format that improves the accuracy and repeatability of cell counting and provides convenient room temperature storage.

Contents and storage

Product	Catalog No.	Amount	Concentration	Size	Excitation	Emission	Storage
CountBright™ Absolute Counting Beads	C36950	5 mL	~0.5 × 10^5 beads/50 µL Refer to bottle for lot-specific concentration.	7 µm	350-635 nm	385-800 nm	Store at 2-8°C. Protect from light.
CountBright™ Plus Absolute Counting Beads	C36995			4 µm	350-810 nm	385-860 nm	
CountBright™ Plus Ready Tubes	C40000	50 tubes	~50,000 beads / tube Refer to product packaging for lot-specific concentration.	4 µm	350-810 nm	385-860 nm	Room Temperature.[1]; Protect from light.

[1] Store in original packaging.

CountBright™ Absolute Counting Beads and CountBright™ Plus Absolute Counting Beads

Before you begin

- Cell count accuracy based on CountBright™ Absolute Counting Beads and CountBright™ Plus Absolute Counting Beads depends on sample handling and precise delivery of the volume of beads. The beads must be mixed well to ensure a uniform suspension of microspheres. This can be achieved by vortexing for 30 seconds before removing an aliquot.
- The microsphere suspension can be pipetted by standard techniques, but more viscous solutions, such as blood, require reverse pipetting for accurate volume delivery.
- Cell suspensions may be diluted, but should be assayed without wash steps after the addition of the beads.

Experimental protocol

Prepare cells for counting

1. Process cells as needed before counting. This may include:
   - Staining cells with fluorochrome-conjugated antibodies
   - Fixation and/or permeabilization of cells
   - Lysis of red blood cells
2. Allow the absolute counting beads to warm to room temperature. Vortex the bead suspension for 30 seconds to completely resuspend.
3. Immediately after vortexing, add 50 µL of the counting bead suspension to each sample to be counted and vortex (the volume of each sample needs to be noted prior to addition of beads).

   Note: Any buffer appropriate for cells (e.g., flow cytometry staining buffer or other buffered saline solutions, lysis buffer, fixation buffer, permeabilization buffer) can be used to bring the volume up to at least 300 µL. This volume is sufficient to dilute the small amount of detergent present in the beads storage buffer.

Run samples on flow cytometer

1. On flow cytometer, set the forward scatter (FSC) threshold low enough to include the beads on the linear-forward scatter vs. the linear-side scatter plot (SSC).
2. Adjust fluorescence detector voltages such that stained cells and the absolute counting beads are appropriately on scale.
3. Use normal gating strategies to identify the cell population to be enumerated.
4. Draw a gate on the absolute counting beads and collect at least 1,000 bead events to ensure a statistically significant determination of sample volume (e.g., Figure 1).

   Note: A fluorescence threshold may also be used to analyze cells and beads.
5. Using the count statistics from these 2 gates, the concentration of the original cell sample may be determined by the equations. See “Calculations” on page 2.

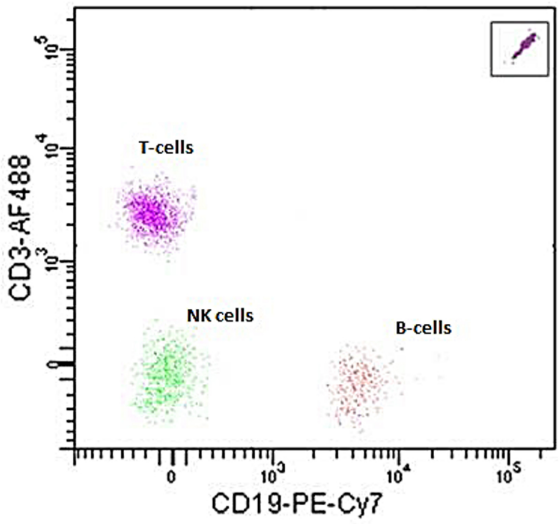

Figure 1 Normal human peripheral blood was stained with Anti-Human CD3 Alexa Fluor™ 488 and Anti-Human CD19 PE-Cy7, then lysed with 1-Step™ Fix/Lyse Solution. CountBright™ Plus Absolute Counting Beads (boxed, purple) were added to the sample prior to collection on a flow cytometer.

Calculations

Note: Only the ratio of the measured volumes of cells and absolute counting beads added to a sample is accounted for in the counting equations. Additional volumes of staining buffer, lysis buffer, etc., do not need to be taken into account when calculating absolute counts. Absolute count is defined as the concentration of cells in the original sample added to the counting tube.

- When cells and absolute counting beads are mixed in a 1:1 ratio of volumes, as in the preceding protocol, the following equation applies:

$$Absolute\ count\ \left(\frac{cells}{\mu L}\right) = \frac{Cell\ count}{Counting\ beads\ count} \times Counting\ beads\ concentration\ from\ bottle\ \left(\frac{beads}{\mu L}\right)$$

- When the cell to bead ratio is not 1:1, use the following equation (Note: Cell volume is the volume noted in Step 3 of the protocol.):

$$Absolute\ count\ \left(\frac{cells}{\mu L}\right) = \frac{(Cell\ count\ \times Counting\ beads\ volume)}{(Counting\ beads\ count\ \times Cell\ volume)} \times Counting\ beads\ concentration\ \left(\frac{beads}{\mu L}\right)$$

  Sample calculation:
  A 1 mL volume of cells was stained. Afterwards, 50 µL of absolute counting beads was added at a bottle concentration of 0.49-0.5 × 10^5 beads/50 µL. The number of cell events was determined to be 1,700; bead events numbered 1,030.

$$\frac{1{,}700\ cells}{1{,}030\ beads} \times \frac{49{,}500\ beads}{1{,}000\ \mu L} = 81.7\ cells/\mu L$$

CountBright™ Plus Ready Tubes

Before you begin

- To ensure accurate cell counts, it is recommended that a lyse/no-wash or no-lyse/no-wash protocol be followed. This ensures that no cells or beads are lost during the washing steps.
- Using precise volumes is critical. It is recommended that blood or other viscous cellular samples are transferred using reverse pipetting.
- While the following workflow is recommended, the format of CountBright™ Plus Ready Tubes makes them amenable to any appropriate cell counting flow cytometry protocol.
- Once an individual pouch of tubes has been opened, it is recommended that the tubes are used immediately.
- Lysis and staining conditions should be optimized prior to carrying out absolute cell counting.

Experimental protocol

Prepare CountBright™ Plus Ready Tubes

1. Inspect the CountBright™ Plus Ready Tubes prior to use to ensure that the small, white bead pellet is intact.

   Note: The tube may be tapped on the benchtop to allow the bead pellet to fall to the bottom of the tube.

2. Pipette sample to be analyzed into the tube. For example, 50 µL of whole blood.
3. Add appropriate reagents to stain the cells.
4. Cap the tube then vortex gently to mix.
5. Incubate as necessary for the reagents used.

(Optional) Lyse/no-wash cells in CountBright™ Plus Ready Tubes

1. Add the appropriate volume of lysis buffer (e.g. 500 µL of eBioscience™ 1X RBC Lysis Buffer).
2. Cap then vortex gently to mix.
3. Incubate as necessary for the lysis reagent used.

(Optional) No-lyse/no-wash cells in CountBright™ Plus Ready Tubes

Add an appropriate volume of buffer appropriate for cells (e.g. flow cytometry staining buffer or PBS) to bring the final volume to at least 500 µL.

Run samples on flow cytometer

1. On flow cytometer, make sure to set the threshold low enough to include the beads on the linear forward scatter by linear side scatter plot.
2. Adjust fluorescence detector voltages such that the stained cells and the CountBright™ Plus beads are appropriately on scale.
3. Use normal gating strategies to identify the cell population to be enumerated.
4. Draw a gate on the CountBright™ Plus beads and collect at least 1000 bead events to ensure a statistically significant determination of cell counts.
5. Using the counts derived from the cell population and bead gates, the concentration of the sample may be determined by the equation. See “Calculations” on page 4.

Calculations

For this example calculation, 50 µL of whole blood was added to a CountBright™ Plus Ready Tubes containing 56,600 beads / tube. The cells were stained with 20 µL of primary antibody conjugates and then lysed with 500 µL of lysis buffer.

$$Absolute\ Count\ \left(\frac{cells}{\mu L}\right) = \frac{Cell\ Count}{Bead\ Count} \times \frac{Total\ Beads}{Sample\ Volume\ (uL)}$$

$$Absolute\ Count\ \left(\frac{cells}{\mu L}\right) = \frac{3792\ cells}{10990\ beads} \times \frac{56{,}500\ beads}{50\ \mu L}$$

$$Absolute\ Count\ = 390\ \frac{cells}{\mu L}$$

Figure 2 Example calculation

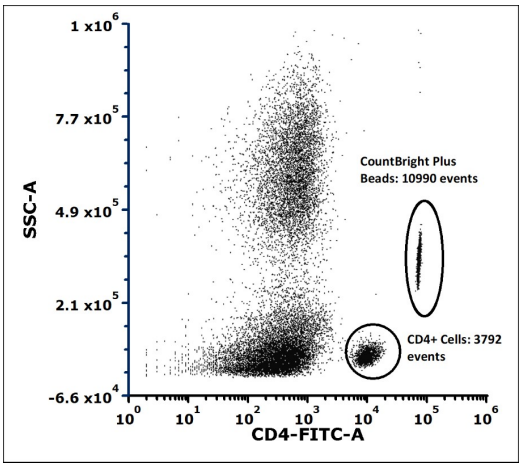

Figure 3 Linear forward scatter by linear side scatter plot

Limited product warranty

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Revision history: Pub. No. MAN0018850

Revision	Date	Description
B.0	13 July 2023	Added new content for CountBright™ Plus Ready Tubes.
A.0	14 October 2019	New document for CountBright™ and CountBright™ Plus Absolute Counting Beads.