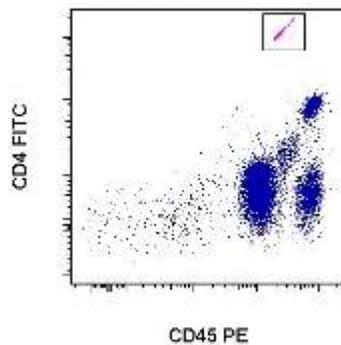


## 123count™ eBeads Counting Beads

Catalog Number: 01-1234

Also known as: Absolute cell count beads

GPR: General Purpose Reagents. For Laboratory Use.



Normal human peripheral blood was stained with Anti-Human CD45 PE (cat. 12-0459) and Anti-Human CD4 FITC (cat. 11-0048) (blue), then lysed with 1-Step Fix/Lyse Solution. 123count eBeads (boxed, purple) were added to the sample prior to collection on a flow cytometer. Total events were used for analysis.

### Product Information

**Contents:** 123count™ eBeads Counting Beads



**Catalog Number:** 01-1234

**Concentration:** Refer to vial

**Formulation:** 0.05% Tween-20 and 2 mM sodium azide

**Temperature Limitation:** Store at 2-8°C protected from light. Do not freeze.

**Batch Code:** Refer to vial

**Use By:** Refer to vial

**Contains sodium azide**



### Description

123count eBeads are intended for use in absolute counting of cells or other particles by flow cytometry. These are 7 µm microparticles containing encapsulated dyes compatible with blue (488 nm) and violet (405 nm) excitation sources and emitting fluorescence between approximately 500 nm and 750 nm. They are supplied at a known concentration in 0.05% Tween-20 with 2 mM sodium azide. Collecting carefully suspended and measured quantities of cell:bead mixtures on a flow cytometer makes the ratiometric enumeration of cells possible.

### Applications Reported

123count eBeads has been reported for use in flow cytometric analysis.

### Applications Tested

123count eBeads has been tested on human peripheral blood. 123count eBeads can be used at 100 µL per test. A test is defined as the amount beads necessary for the enumeration of a 100 µL cell sample.

### References

Perdu S, Castellana B, Kim Y, Chan K, DeLuca L, Beristain AG. Maternal obesity drives functional alterations in uterine NK cells. JCI Insight. 2016 Jul 21;1(11):e85560. (123count eBeads, FC, PubMed)

### Related Products

00-4222 eBioscience™ Flow Cytometry Staining Buffer

00-5333 eBioscience™ 1-step Fix/Lyse Solution (10X)

11-0048 eBioscience™ Anti-Human CD4 FITC (OKT4 (OKT-4))

12-0459 eBioscience™ Anti-Human CD45 PE (HI30)

Not for further distribution without written consent.

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# 123count eBeads

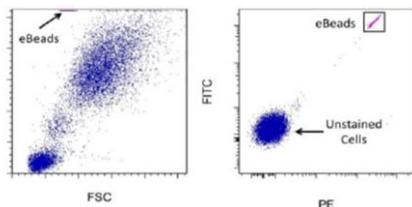
## Introduction

123count eBeads are intended for use in absolute counting of cells or other particles by flow cytometry. These are 7  $\mu\text{m}$  microparticles containing encapsulated dyes compatible with blue (488 nm) and violet (405 nm) excitation sources and emitting fluorescence between approximately 500 nm and 750 nm. They are supplied at a known concentration in 0.05% Tween-20 with 2 mM sodium azide. Collecting carefully suspended and measured quantities of cell:bead mixtures on a flow cytometer makes the ratiometric enumeration of cells possible.

## General Notes

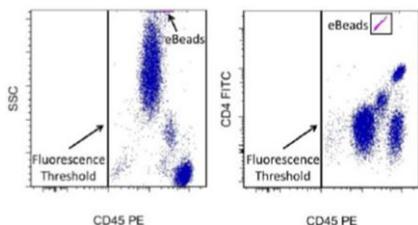
### Physical Characteristics of 123count eBeads

1. 123count eBeads are a suspension containing approximately 1000 beads/ $\mu\text{L}$ . Refer to vial label for lot-specific concentration to be used in absolute count calculations.
2. 123count eBeads settle out of suspension over time. Store upright at 2-8°C in the dark. Mix by vortexing or inverting for 15-30 seconds before use.
3. 123count eBeads are supplied in a buffer containing Tween-20. Cell samples should be in a total volume of at least 300  $\mu\text{L}$  before addition of 100  $\mu\text{L}$  of 123count eBeads. This volume sufficiently dilutes Tween-20 to avoid damage to cell samples.
4. 123count eBeads have properties resulting in low forward scatter. Be sure beads are above forward scatter threshold and adjust FSC voltages if necessary.
5. 123count eBeads have higher side scatter than many cell populations. Use two fluorescence parameters excited by the blue (488 nm) or violet (405 nm) laser line instead of light scatter parameters when identifying these beads (see example below).
6. 123count eBeads contain encapsulated dyes that are excited by both blue (488 nm) and violet (405 nm) lasers. They emit bright fluorescence between 500-750 nm. They can be detected as a compact, bright population in detectors including those designated for blue-excited FITC, PE, PE-eFluor™ 610, PerCP-eFluor™ 710, and PE-Cyanine7, and violet-excited eFluor™ 450, eFluor™ 506, eVolve™ 605, and eVolve™ 655. 123count eBeads do not contain dyes excited by red (633- 640 nm) lasers. The optimal detector(s) for identification of 123count eBeads may require optimization in a given antibody staining panel.



*Due to 123count eBeads' high side scatter properties, it is better to identify them by fluorescence. Human lysed whole blood was mixed with 123count eBeads and then collected on a flow cytometer. Left: 123count eBeads fall off-scale for side scatter when lysed whole blood settings are used. Right: 123count eBeads are easily visualized with fluorescent parameters such as FITC and PE.*

7. If applying a fluorescence threshold instead of a FSC threshold, use a channel in which 123count eBeads are positive. (See example below.)



*Use fluorescence thresholds only in channels where 123count eBeads are positive. Human lysed whole blood was stained with CD45 PE and CD4 FITC and then 123count eBeads were added before analysis. An electronic threshold of 1000 on the PE detector was used during analysis.*

## Accuracy of Measurements

1. Accuracy of counts is dependent on accuracy in two critical pipetting steps: (1) addition of the cell sample and (2) addition of 123count eBeads. Use calibrated pipets appropriate for the measurement of 100  $\mu\text{L}$ . If a given experiment necessitates the measurement of a different volume of cells or eBeads, use a pipet appropriate for that volume.
2. Be sure to mix both cells and 123count eBeads well before pipetting.
3. Centrifugation/wash steps should be avoided or kept to a minimum, as each manipulation of the sample will reduce the accuracy of the resultant count.

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4. Reproducibility of counts is dependent on sample mixing and pipetting accuracy. Given good technique, CV values of 6-10% should be attainable.

### Calculations

1. Only the ratio of the measured volumes of cells and eBeads 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.
2. When cells and eBeads are mixed at 1:1 ratio of volumes, as in the below protocol, use the following equation for calculation of absolute counts:

$$\text{Absolute Count (cells/}\mu\text{L)} = \frac{\text{Cell Count}}{\text{eBead Count}} \times \text{eBead Concentration}$$

3. If an experiment requires the use of different volumes of cells and eBeads (not 1:1), use the following equation to incorporate these differences:

$$\text{Absolute Count (cells/}\mu\text{L)} = \frac{(\text{Cell Count} \times \text{eBead Volume})}{(\text{eBead Count} \times \text{Cell Volume})} \times \text{eBead Concentration}$$

*Note: Cell and eBead counts are obtained from gating strategies used during analysis. See the Experimental Procedure below for more details.*

*Note: Refer to vial label for lot-specific concentration for use in absolute count calculations.*

### Protocol

#### Step I: Sample Preparation

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

*Note: Accurate counting results are dependent on careful measurement of the volumes of cells and beads to be counted. Use calibrated pipets.*

*Note: Manipulation of samples such as centrifugation and decanting or transferring from one tube to another may reduce the accuracy of counts. These manipulation steps should be minimized as much as possible.*

2. Vortex 123count eBeads for 15-30 seconds to ensure uniform mixing.

*Note: Uniform mixing is critical for accurate counting results.*

3. For sufficient dilution of 123count eBeads storage buffer, ensure that each cell sample contains at least 300  $\mu\text{L}$  of total volume prior to addition of 123count eBeads.

*Note: Any buffer appropriate for cells (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  $\mu\text{L}$ . This volume is sufficient to dilute the small amount of detergent present in the 123count eBeads storage buffer. The exact volume of any additional buffer is not critical to the accuracy of counting.*

4. Add 100  $\mu\text{L}$  of 123count eBeads to each sample to be counted.

*Note: Include one tube that contains only staining buffer and 100  $\mu\text{L}$  of 123count eBeads and one sample that contains stained cells but not 123count eBeads. These two samples may be useful for cytometer setup.*

#### Step II: Cytometer Setup

5. Set Forward Scatter (FSC) and Side Scatter (SSC) voltages so that cells can be appropriately visualized. This can be done with an unstained sample with or without 123count eBeads added. Fluorescence detectors can be adjusted to locate cells appropriately on scale at this time.
6. Check that 123count eBeads are above FSC threshold. This can be done with the eBeads-alone sample mentioned in step 4 or with any sample containing eBeads. Adjust FSC and threshold settings if necessary. See General Notes 3-6 above for more information.

*Note: 123count eBeads have characteristics resulting in low FSC and high SSC. They can be identified as a compact, bright population in any blue (488 nm)- or violet (405 nm)-excited fluorescence detector. If they are not easily identifiable by fluorescence, they may be below FSC threshold.*

*Note: Due to 123count eBeads high SSC properties, it may not be possible to adjust voltages to visualize both eBeads and cells in SSC. Therefore, SSC voltages should be set for the optimal visualization of cells.*

7. Adjust fluorescence detector voltages such that stained cells and 123count eBeads are appropriately on scale. 123count eBeads are brightly fluorescent in all blue (488 nm) and violet (405nm) laser-excited detectors.

8. Run single-color compensation controls and perform compensation setup.

*Note: Single color compensation controls should not contain 123count eBeads.*

9. Collect samples on flow cytometer. Briefly vortex (~5 seconds) each tube immediately before collection to ensure equal dispersion of cells and beads.

### **Step III: Data Analysis and Absolute Count Determination**

10. Use normal gating strategies to identify the cell population to be enumerated (example: FSC/SSC lymphocyte gate → CD3+CD4+ gate)

11. In the same sample, draw a gate on 123count eBeads in an ungated plot displaying two blue (488 nm) or violet (405 nm) laser-excited parameters.

12. Using the count statistics from these two gates, the concentration of the original cell sample may be determined by the equations listed in the General Notes section above.

*Note: At least 1000 beads should be collected from every sample to ensure statistically significant results.*

## Documentation and support

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  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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23 January 2017

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