BD Trucount™ Controls

To control certain elements of the absolute counting process

30 Tests—Catalog No. 664343

23-22530(03)
2023-08
English

℞ Only IVD

1. INTENDED USE

BD Trucount™ Controls are designed for use with BD Trucount™ Tubes and a suitably equipped flow cytometer as a control for certain elements of the absolute counting process. Specifically, a control bead value that is outside the expected range could indicate an error in pipetting or a problem with the value from the BD Trucount™ beads. BD Trucount™ Controls are not intended as a substitute for a cellular control.

2. PRINCIPLES OF THE PROCEDURE

BD Trucount™ Controls comprise three vials of control beads, each with a different concentration – low, medium, or high. The control bead suspensions are added to normal blood in BD Trucount™ Tubes. For BD FACSCalibur™ flow cytometers, the blood is stained first with appropriate antibody reagents.

If the appropriate cytometer-specific BD analysis software (see Table 1) is used, the control bead count will be determined by the software. You can also manually perform data analysis using BD CellQuest™ Pro software, for example.

3. REAGENT

Reagent provided, sufficient for 30 tests.

Concentration values are listed in the following table:

BD Trucount™ Controls	Concentration (beads/mL)
Low Control Beads	4.72×10^4–5.25×10^4
Medium Control Beads	2.351×10^5–2.635×10^5
High Control Beads	9.403×10^5–1.0539×10^6

Precautions

- The addition of a precise volume of control beads is critical to achieve the intended result. Pipettes must be calibrated to deliver exactly 50 µL of sample. An electronic pipette which operates in the reverse pipetting mode is available through BD. If this or a similar pipette is not used, perform the reverse pipetting technique (see Reverse Pipetting in Section 6 for a brief description). Refer to the pipette manufacturer's instructions for more information.

- Always be sure to use the bead count from the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating an absolute count. The correct bead count is critical to determine a cell count. Do not mix multiple lots of tubes in the same run.
- BD Trucount™ Controls are designed for use with a specific lyse/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.
- BD Trucount™ Controls are sensitive to compensation, specifically in FL2–%FL1. If lyse/no-wash procedures are not employed, care must be taken to ensure FL2 brightness is adequate.
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves. Fixation has been reported to inactivate HIV.[3]

Storage and Handling

Store at 2–8 °C. Do not use after the expiration date on the vial.

4. INSTRUMENTS

BD Trucount™ applications are designed for flow cytometers equipped with appropriate computer hardware and software. BD has developed cytometer-specific software that can set photomultiplier tube (PMT) voltages and fluorescence compensation, check instrument sensitivity and performance, or perform daily quality control. BD has also developed software that automatically calculates absolute counts when BD Trucount™ Tubes are used. However, other software packages manufactured by companies other than BD can be used for data acquisition and analysis, and absolute counts can be calculated manually. We recommend the BD systems listed in Table 1 for cytometer setup, acquisition, and analysis. See the corresponding reagent or cytometer IFUs for details.

Results can be achieved using other platforms. The flow cytometer must be equipped to detect at least three-color fluorescence, forward scatter (FSC), and side scatter (SSC). Users of flow cytometers manufactured by companies other than BD should refer to the manufacturer's instructions for setting up three-, four-, and six-color immunophenotyping.

The BD FACS™ Loader, the BD FACSVia™ Loader, and the BD FACS™ Universal Loader can also be used with this product.

Table 1 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™	BD® CS&T Beads BD® FC Beads 7-Color Kit	BD FACSuite™ Clinical application	BD FACSuite™ Clinical application
BD FACSVia™	BD® CS&T Beads	BD FACSVia™ clinical software	BD FACSVia™ clinical software
BD FACSCalibur™	BD Calibrite™ 3-Color Kit BD Calibrite™ APC Beads	BD FACSComp™ software	BD Multiset™ software

5. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture[4,5] into a sterile (lavender top) BD Vacutainer® EDTA blood collection tube or equivalent. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20–25 °C) until ready for staining.

6. PROCEDURE

Reagent Provided

BD Trucount™ Controls (Catalog No. 664343). Provided as three vials of low, medium, and high concentration suspensions of fluorescent beads.

Reagents and materials required but not provided

- BD Trucount™ Tubes (Catalog No. 340334)
- For BD FACSLyric™ flow cytometers:

  BD® CS&T Beads (Catalog Nos. 662413, 662414)

  BD® FC Beads 7-Color Kit (Catalog No. 662961)

  NOTE Use only BD®FC Beads Dilution Buffer, supplied with the kit, to reconstitute the BD® FC Beads.
- For BD FACSVia™ flow cytometers:

  BD® CS&T Beads (Catalog Nos. 662413, 662414)

  Filtered deionized (DI) water

  NOTE For the BD FACSVia™ flow cytometer, use only filtered DI water to dilute BD® CS&T Beads.
- For BD FACSCalibur™ flow cytometers:

  BD Calibrite™ 3-Color Kit and BD Calibrite™ APC Beads

  Consult your BD representative or refer to your product catalog for information on the specific BD Calibrite™ product for your application.
- BD FACSFlow™ Sheath Fluid (Catalog No. 342003) or equivalent

  NOTE Use only BD FACSFlow™ Sheath Fluid to dilute BD Calibrite™ 3-Color Kit, BD Calibrite™ APC Beads, and BD® CS&T Beads.
- BD FACS™ Lysing Solution (10X), 100 mL (Catalog No. 349202)

  See the BD FACS™ Lysing Solution IFU for precautions and warnings.
- Reagent grade (distilled or deionized) water
- BD Vacutainer® EDTA blood collection tubes, or equivalent
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing 1X BD FACS™ Lysing Solution

Diluting BD FACS™ Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

Reverse Pipetting

A precise volume of control beads is critical. If the BD electronic pipette or a similar pipette that delivers a precise volume is not used, perform reverse pipetting.

For reverse pipetting, depress the button to the second stop. Release the button to draw excess sample into the tip. Press the button to the first stop to expel a precise volume of sample, leaving excess sample in the tip.

Preparing the Controls (BD FACSLyric™ and BD FACSVia™ flow cytometers)

1. Remove three BD Trucount™ Tubes from the foil pouch. Label the tubes Low, Medium, and High.

   NOTE Before use, verify that the BD Trucount™ bead pellet is intact and within the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount™ Tube and replace it with another.

   NOTE Use care to protect the tubes from direct light. Perform the procedure at room temperature (20–25 °C).
2. Gently vortex each control vial for 30 seconds and add 50 µL of the low control beads to the tube labeled Low, 50 µL of the medium control beads to the tube labeled Medium, and 50 µL of the high control beads to the tube labeled High.

   NOTE Do not add antibody reagent.
3. Pipette 50 µL of well-mixed, anticoagulated whole blood from a hematologically normal donor onto the side of the tube just above the retainer.
4. Add 450 µL of 1X BD FACS™ Lysing Solution to each tube. Cap the tubes and vortex gently to mix.

The samples are now ready to be analyzed on the flow cytometer.

Staining Blood and Preparing the Controls (BD FACSCalibur™ flow cytometer)

Refer to the appropriate reagent IFU for detailed instructions on sample preparation.

1. Remove three BD Trucount™ Tubes from the foil pouch. Label the tubes Low, Medium, and High.

   NOTE Before use, verify that the BD Trucount™ bead pellet is intact and within the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount™ Tube and replace it with another.
2. Pipette 20 µL of the appropriate antibody reagent just above the stainless steel retainer. Do not touch the pellet.

   NOTE Use care to protect the tubes from direct light. Perform the procedure at room temperature (20–25 °C).
3. Pipette 50 µL of well-mixed, anticoagulated whole blood from a hematologically normal donor onto the side of the tube just above the retainer.

4. Cap the tubes and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature (20–25 °C).
5. Add 450 µL of 1X BD FACS™ Lysing Solution to each tube.
6. Cap the tubes and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature.
7. Gently vortex each control vial for 30 seconds and add 50 µL of the low control beads to the tube labeled Low, 50 µL of the medium control beads to the tube labeled Medium, and 50 µL of the high control beads to the tube labeled High.

The samples are now ready to be analyzed on the flow cytometer.

7. FLOW CYTOMETRY

- Vortex the samples thoroughly (at low speed) to resuspend beads and reduce cell aggregation before running them on the flow cytometer.[6]
- If using the Loader for acquisition, vortex tubes immediately before placing them into the Loader racks.
- Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.
- Acquire and analyze data using the appropriate cytometer-specific BD software.

8. RESULTS

If you are not using a BD software program that automatically calculates absolute counts, you can perform a manual calculation for the low, medium, and high control beads using the following equation:

A = B/C × D/E where:

A = absolute count of BD Trucount™ Control beads

B = number of events in region containing BD Trucount™ Control beads

C = number of events in BD Trucount™ bead region

D* = number of beads per test

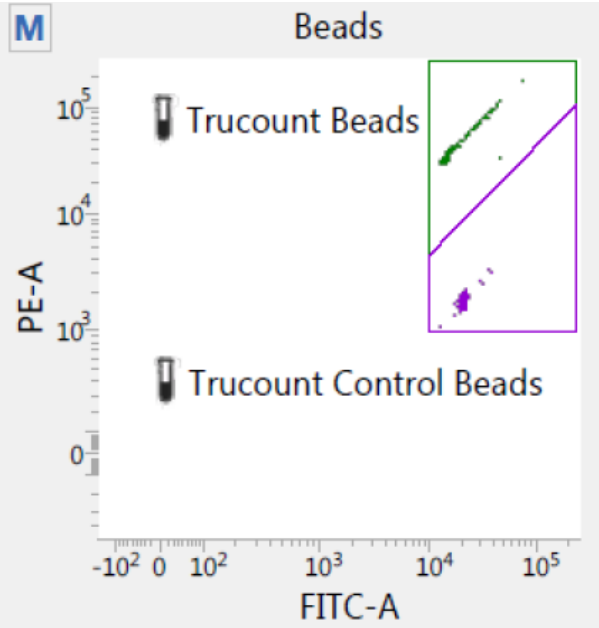
E = test sample volume (50 µL)

*This value is found on the BD Trucount™ Tubes foil pouch label and might vary from lot to lot.

Obtain the number of events in the BD Trucount™ Control bead region and the BD Trucount™ bead region from the statistics of an FITC-A (FL1) vs PE-A (FL2) dot plot gated on all beads.

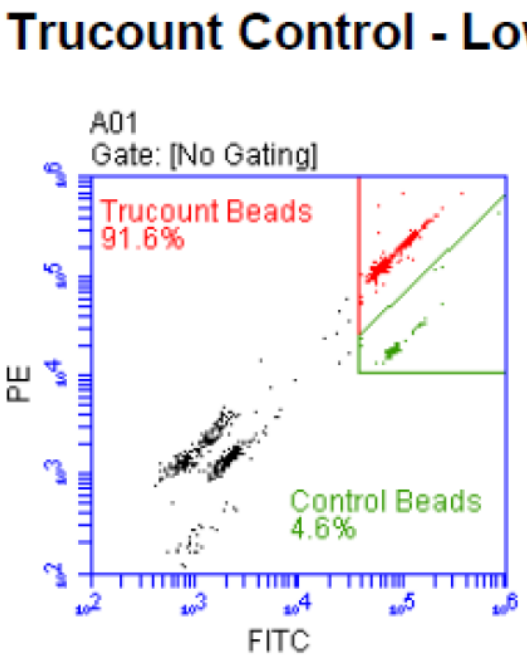
The dot plot shown in Figure 1 was acquired with BD FACSuite™ Clinical application on a BD FACSLyric™ flow cytometer.

Figure 1 Example of a FITC-A (FL1) vs PE-A (FL2) dot plot showing BD Trucount™ Beads and BD Trucount™ Control Beads



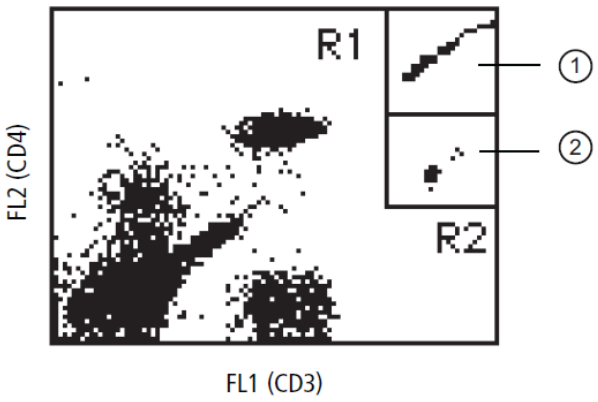
The dot plot shown in Figure 2 was acquired on a BD FACSVia™ flow cytometer.

Figure 2 Example of a FITC (FL1) vs PE (FL2) dot plot showing BD Trucount™ Beads and Control Beads



The dot plot shown in Figure 3 was acquired on a BD FACSCalibur™ flow cytometer.

Figure 3 Example of ungated FL1 (CD3) vs FL2 (CD4) dot plot showing BD Trucount™ bead (1) and BD Trucount™ Control bead (2) regions



The control beads box label contains expected target ranges for the low, medium, and high bead counts. These ranges vary from lot to lot. If the values obtained are outside the expected range, pipetting imprecision or other errors in the process are suspect.

9. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometer

Accuracy and precision of system performance of the BD FACSLyric™ flow cytometer with BD Trucount™ Controls were evaluated in 4 separate 20-replicate runs prepared from 1 of 3 donors, by 1 of 3 operators, using 1 of 4 lots of BD Trucount™ Controls (Low, Medium, or High), and acquired on 1 of 4 BD FACSLyric™ flow cytometers. The mean bead count for each lot is compared to the expected count printed on the box label to determine accuracy, and the standard deviation (SD) of each level is evaluated for precision. The mean, mean %bias, and SD results for each lot of control beads are shown in Table 2.

Table 2 Control bead absolute counts vs expected counts (BD FACSLyric™ flow cytometer)

Level	Lot No.	N, reps	Mean (beads/µL)	Mean %Bias	SD
Low	1	20	50.5	3.06	7.8
	2	20	52.1	4.10	8.1
	3	20	54.4	6.67	8.2
	4	20	51.7	7.71	7.7
Medium	1	20	260.3	5.81	21.4
	2	20	260.0	1.54	22.2
	3	20	259.4	1.73	22.2
	4	20	256.6	4.71	21.3

Level	Lot No.	N, reps	Mean (beads/µL)	Mean %Bias	SD
High	1	20	1,002.3	2.17	63.8
	2	20	1,054.1	2.44	66.9
	3	20	1,050.9	2.53	66.6
	4	20	1,029.8	4.87	63.9

BD FACSVia™ Flow Cytometer

Accuracy and precision of system performance of the BD FACSVia™ system with BD Trucount™ Controls were evaluated in 3 separate 20-replicate runs prepared from 1 of 3 donors, by 1 of 3 operators, using 1 of 3 lots of BD Trucount™ Controls (Low, Medium, or High), and acquired on 1 of 3 BD FACSVia™ cytometers. The mean bead count for each lot is compared to the expected count printed on the box label to determine accuracy, and the SD of each level is evaluated for precision. The mean, mean %bias, and SD results for each lot of control beads are shown in Table 3.

Table 3 Control bead absolute counts vs expected counts (BD FACSVia™ flow cytometer)

Level	Lot No.	N, reps	Mean (beads/µL)	Mean %Bias	SD
Low	1	20	51.95	1.86	3.20
	2	20	51.55	7.40	3.50
	3	20	50.75	3.57	2.47
Medium	1	20	247.70	–3.62	14.21
	2	20	237.30	–3.14	6.77
	3	20	246.05	0.02	7.96
High	1	20	996.00	–3.11	46.92
	2	20	952.95	–2.96	28.72
	3	20	981.70	0.07	18.30

BD FACSCalibur™ Flow Cytometer

Note that BD Trucount™ Controls performance using the BD FACSCalibur™ flow cytometer is shown with BD Tritest™ CD3/CD4/CD45 as an example. Other IVD BD Tritest™ reagents have similar performance using the BD FACSCalibur™ flow cytometer.

Accuracy

Control bead counts were determined with BD Trucount™ Tubes and BD Tritest™ CD3/CD4/CD45 for two donors. The control bead count was compared to the expected count determined from the concentration printed on the label. Three replicates per each bead level were run, and results were compared using regression analysis. Results are shown in Table 4.

Table 4 Accuracy: BD Trucount™ Control beads

Donor	Parameter		
	R^2	Slope	Intercept
1	0.992	1.047	–4.719
2	0.998	1.084	–8.184

Precision

Precision was measured using three lots of BD Trucount™ Controls on a single donor, with a single lot of BD Trucount™ Tubes and the BD Tritest™ CD3/CD4/CD45 reagent. The mean, SD, and %CV (coefficient of variation) were computed for each lot. Results are shown in Table 5.

Table 5 Precision: BD Trucount™ Control beads

Level	Lot #	N, reps	Mean (cells/µL)	SD	%CV
Low	1	18	49.45	6.86	13.87
	2	18	47.74	8.46	17.72
	3	18	50.15	8.40	16.75
Medium	1	18	241.31	22.03	9.13
	2	18	240.93	17.37	7.21
	3	18	260.07	16.78	6.45
High	1	18	940.62	44.46	4.73
	2	18	976.70	44.60	4.57
	3	18	1,004.02	52.93	5.27

REFERENCES

1. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.
2. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
3. Nicholson JK, Browning SW, Orloff SL, McDougal JS. Inactivation of HIV-infected H9 cells in whole blood preparations by lysing/fixing reagents used in flow cytometry. *J Immunol Methods.* 1993;160:215-218.
4. *Collection of Diagnostic Venous Blood Specimens*, *7th ed.* Wayne, PA: Clinical and Laboratory Standards Institute; 2017. CLSI document GP41.
5. *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.
6. Jackson AL, Warner NL. Preparation, staining, and analysis by flow cytometry of peripheral blood leukocytes. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology; 1986:226-235.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

Symbols Glossary

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
#	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
Rx Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
12	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
/	<Box #> / <Total Boxes>
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

Note: Text layout in symbols is determined by label design.

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HISTORY

Revision	Date	Changes made
23-22530(03)	2023-08	Updated legal manufacturer address. Updated symbols glossary. Added History section. Updated Patents and Trademarks section.