# Certificate

Management system as per

## ELOT EN ISO 9001 : 2015

The Certification Body TÜV HELLAS (TÜV NORD) S.A. hereby confirms as a result of the audit, assessment and certification decision according to ISO/IEC 17021-1:2015, that the organization:

**BECTON DICKINSON HELLAS S.A.**
**1, Filellinon Str. & M. Alexandrou Str.**
**164 52 Argyroupoli**
**Hellas**

operates a management system in accordance with the requirements of ELOT EN ISO 9001 : 2015 and will be assessed for conformity within the 3 year term of validity of the certificate.

Scope

**Distribution and Sales of Interventional Medical Devices (Surgical, Cardiovascular, Vascular, Endovascular, Urological, Biopsy, Brachytherapy, Endoscopic and Critical Care) and Accessories, Non - Invasive Temperature Management Systems, In - Vitro Diagnostic Medical Devices (Specimen Radiography Systems) and Technical Support of Biopsy and Imaging Systems, Non - Invasive Temperature Management Systems, Endovascular Medical Devices, Vascular Embolisation Devices and Vascular Guidance and Positioning Systems.**

Certificate Registration No. 041 21 0020
Audit Report No. E- 3482/2024

Valid from 2024-03-01
Valid until 2027-02-28
Initial certification 2021

Athens, 28.02.2024

TÜV HELLAS (TÜV NORD) S.A. Certification Body

Digitally signed by Bolea Petru
Date: 2024.08.26 15:28:46 EEST
Reason: MoldSign Signature
Location: Moldova

**TÜV HELLAS (TÜV NORD) S.A.**
282, Mesogeion Ave.
155 62 Athens, Greece
tuvhellas.gr

MS Certification
No of certificate 185

TÜV ®

TÜVNORDGROUP

# Certificate of Registration

## QUALITY MANAGEMENT SYSTEM - ISO 13485:2016 & EN ISO 13485:2016

This is to certify that:

Becton, Dickinson and Company (BD)
7 Loveton Circle
Sparks
Maryland
21152
USA

Holds Certificate Number: **MD 595740**

and operates a Quality Management System which complies with the requirements of ISO 13485:2016 & EN ISO 13485:2016 for the following scope:

The design, development, manufacture, service and distribution of in-vitro diagnostic medical devices and microbiological products. These devices and products include equipment, in-vitro diagnostic test kits and reagents, prepared media products, dehydrated culture media, collection and transport, antimicrobial susceptibility tests, sample preparation, cytology devices, cytopathology auto-imaging devices with computerized microscopy, telepathology devices, lab automation, ancillary devices and instrument software for use in the screening and diagnosis of diseases, transmissible and sexually transmissible agents, and autoimmune status.

For and on behalf of BSI:

Gary E Slack, Senior Vice President - Medical Devices

Original Registration Date: 2013-03-14
Latest Revision Date: 2021-10-08

Effective Date: 2021-10-11
Expiry Date: 2024-10-10



...making excellence a habit.™

Certificate No: **MD 595740**

| Location | Registered Activities |
|---|---|
| Becton, Dickinson and Company (BD)<br>7 Loveton Circle<br>Sparks<br>Maryland<br>21152<br>USA | The design, development, manufacture, service and distribution of in-vitro diagnostic medical devices and microbiological products. These devices and products include equipment, in-vitro diagnostic test kits and reagents, prepared media products, dehydrated culture media, collection and transport, antimicrobial susceptibility tests, sample preparation, cytology devices, cytopathology auto-imaging deviceswith computerized microscopy, telepathology devices, lab automation, ancillary devices and instrument software for use in the screening and diagnosis of diseases, transmissible and sexually transmissible agents, and autoimmune status. |
| Becton Dickinson and Company (BD)<br>BD Diagnostic Systems<br>52/54 Loveton Circle<br>Sparks<br>Maryland<br>21152<br>USA | The design, development, manufacture, service and distribution of in-vitro diagnostic medical devices and microbiological products. These devices and products include equipment, in-vitro diagnostic test kits and reagents used in the diagnosis of diseases, transmissible and sexually transmissible agents, autoimmune status, prepared media products, dehydrated culture media, collection and transport, sample preparation. |
| Becton Dickinson and Company (BD)<br>BD Diagnostic Systems<br>39 Loveton Circle<br>Sparks<br>Maryland<br>21152<br>USA | The design, development, manufacture, service and distribution of in-vitro diagnostic medical devices and microbiological products. These devices and products include equipment, in-vitro diagnostic test kits and reagents used in the diagnosis of diseases, transmissible and sexually transmissible agents, autoimmune status, dehydrated culture media, collection and transport, sample preparation. |
| Becton Dickinson and Company (BD)<br>BD Diagnostic Systems<br>250 Schilling Circle<br>Cockeysville<br>Maryland<br>21030<br>USA | The design, development, manufacture, service and distribution of in-vitro diagnostic medical devices and microbiological products. These devices and products include equipment, in-vitro diagnostic test kits and reagents used in the diagnosis of diseases, prepared media products, collection and transport, antimicrobial susceptibility tests, sample preparation. |

Original Registration Date: 2013-03-14

Effective Date: 2021-10-11

Latest Revision Date: 2021-10-08

Expiry Date: 2024-10-10

# BD Multitest™ CD3/CD8/CD45/CD4

50 Tests per kit—Catalog No. 342417

50 Tests per kit with
BD Trucount™ Tubes—Catalog No. 342447

23-5351(12)
2023-07
English

CE 2797 IVD

## 1. INTENDED USE

The BD Multitest™ CD3/CD8/CD45/CD4 reagent with optional BD Trucount™ Tubes is a four-color direct immunofluorescence reagent for use in identifying and determining the percentages and absolute counts of T cells, as well as the CD4 and CD8 subpopulations of T cells, in peripheral blood on a BD flow cytometer equipped with the following:

- At least a 488-nm blue laser and a 640-nm red laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least 4-color fluorescence
- Software to acquire and analyze the data

### Clinical Applications

Determining percentages or absolute counts of $CD3^+CD4^+$ T lymphocytes is used in monitoring human immunodeficiency virus (HIV)-infected individuals. Individuals with HIV typically exhibit a steady decrease of $CD3^+CD4^+$ T lymphocyte absolute counts as the infection progresses.[1]

Determining percentages or absolute counts of $CD3^+$, $CD3^+CD4^+$, or $CD3^+CD8^+$ T lymphocytes is used to characterize or monitor some forms of immune deficiency and autoimmune diseases.[1,2]

## 2. SUMMARY OF THE TEST

Human peripheral blood contains three types of lymphocytes: T, B, and NK lymphocytes. They have distinct biologic functions and can be identified by differences in their cell-surface antigen expression.

Subsets of antigen-specific T lymphocytes have different roles in the adaptive immune response. Helper/inducer T lymphocytes secrete cytokines that help regulate the activity of other T lymphocytes as well as B lymphocytes. Suppressor/cytotoxic T lymphocytes suppress the activity of other T lymphocytes, or recognize and lyse infected or abnormal cells.[3]

BD Multitest™ CD3/CD8/CD45/CD4 with or without BD Trucount™ Tubes is a quantitative assay intended for use by laboratory professionals to identify and enumerate the following T-lymphocyte subset populations:

- $CD3^+$ T lymphocytes
- $CD3^+CD4^+$ helper/inducer T lymphocytes
- $CD3^+CD8^+$ suppressor/cytotoxic T lymphocytes

Automated sample preparation and acquisition can be achieved using the BD FACSDuet™ Sample Preparation System and BD loaders, respectively. Data analysis can be performed using a pre-defined template and automated gating, which can be manually adjusted by the user, if needed.

## Principle of Operation

The BD Multitest™ CD3/CD8/CD45/CD4 reagent is composed of four monoclonal antibodies, each conjugated to a specific fluorochrome. The reagent is added to peripheral blood and incubated, allowing each monoclonal antibody in the reagent to bind to a specific antigen on the surface of the cells. After incubation, BD FACS™ Lysing Solution is added to lyse the red blood cells in the sample. Cells are acquired on a BD flow cytometer using the appropriate software. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Multitest™ reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. The software and the BD Multitest™ 4-Color assay module are used to analyze the data and report the result.

When determining absolute cell counts, expressed as the number of cells/µL, a precise volume of specimen and the BD Multitest™ CD3/CD8/CD45/CD4 reagent are added to a BD Trucount™ Tube. The BD Trucount™ Tube contains a lyophilized pellet of fluorescent beads. During incubation of the reagent and the specimen, the bead pellet dissolves, releasing a known number of fluorescent beads, which are distinguished from cells by their fluorescence intensity. After lysing red blood cells, the sample is acquired on a BD flow cytometer. The software determines the absolute cell counts by comparing cellular events to bead events, and reports the absolute cell counts in the lab report.

For flow cytometer principles of operation, see the instructions for use (IFU) for your instrument.

# 3. REAGENT

## Reagent Composition

The reagent contains the following conjugated antibodies:

**Table 1** Reagent composition

| Antibody | Fluorochrome | Clone | Isotype | Concentration (µg/mL) |
|---|---|---|---|---|
| CD3 | FITC | SK7[4,5] | $IgG_{1,\kappa}$ | 2.3 |
| CD8 | PE | SK1[6,7] | $IgG_{1,\kappa}$ | 1.75 |
| CD45 | PerCP | 2D1 [8] | $IgG_{1,\kappa}$ | 7.50 |
| CD4 | APC | SK3[6,7,9] | $IgG_{1,\kappa}$ | 0.92 |

CD3 (SK7) recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[10] The CD3 antigen is present on T lymphocytes and is noncovalently associated with either α/β or γ/δ TCR.[11] CD3 reacts minimally with other cell populations.[12]

CD8 (SK1) recognizes an antigen that interacts with class I major histocompatibility complex (MHC) molecules, resulting in increased adhesion between the $CD8^+$ T lymphocytes and the target cells and enhanced activation of resting T lymphocytes.[13,14,15] The CD8 antigen is present on suppressor/cytotoxic T lymphocytes. CD8 also recognizes a subset of NK lymphocytes.[16]

CD45 (2D1) recognizes all isoforms of the leucocyte common antigen (LCA)/T200 family.[17] The CD45 antigen is present on all human leucocytes, including lymphocytes, monocytes, granulocytes, eosinophils, and basophils in peripheral blood.[17] CD45 has been reported to react weakly with mature circulating erythrocytes and platelets.[17,18]

CD4 (SK3) recognizes an antigen that interacts with class II MHC molecules and is the primary receptor for HIV.[19,20] The CD4 antigen is present on helper/inducer T lymphocytes and is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.[9]

### Precautions

- The reagent should be clear. Do not use the reagent if you observe any change in appearance. Precipitation, cloudiness, or change in color indicates instability or deterioration.
- The antibody reagent contains sodium azide as a preservative. However, take care to avoid microbial contamination, which can cause erroneous results.
- If using BD Trucount™ Tubes, calibrate pipets to deliver exactly 50 µL of sample or perform the reverse pipetting technique (see Reverse Pipetting on page 7). See the pipet manufacturer's instructions for more information.
- Bead count varies by lot of BD Trucount™ Tubes. It is critical to use the bead count shown on the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of BD Trucount™ Tubes in the same run.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

### Storage and Handling

- Store the reagent at 2–8 °C. Reagent in opened or unopened vials is stable until the expiration date shown on the vial label. Do not use after this expiration date.
- Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the reagent vial dry.
- The reagent is stable if kept in the BD FACSDuet™ instrument for 8 hours per day for 5 days. Do not store the reagent overnight in the instrument. Use of any reagent remaining after being kept in the BD FACSDuet™ instrument for 5 days must be validated by the user.
- Store BD Trucount™ Tubes in their original foil pouch at 2–25 °C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. Do not remove the desiccant pack from the pouch. Use tubes within 1 hour after removal from the foil pouch.
- BD Trucount™ Tubes in an unopened pouch are stable until the expiration date shown on the packaging. Do not use tubes after the expiration date.
- Tubes in an opened pouch are stable for 1 month after the date of opening, when stored as directed. Write the date when you first open the pouch in the space provided on the label.

## 4. INSTRUMENT

The BD FACSLyric™ and BD FACSCanto™ II systems are outlined in the following table. See the corresponding reagent or instrument user documentation for details.

**Table 2** BD FACSLyric™ and BD FACSCanto™ II systems

| Flow cytometer | Setup beads | Setup software | Analysis software | Assay module |
|---|---|---|---|---|
| BD FACSLyric™ | BD® CS&T Beads[a]<br>BD® FC Beads 7-Color Kit[b] | BD FACSuite™ Clinical application | BD FACSuite™ Clinical application | BD Multitest™ 4-Color |
| BD FACSCanto™ II | BD FACS™ 7-Color Setup Beads[c] | BD FACSCanto™ Clinical Software v2.4 or later | BD FACSCanto™ Clinical Software v2.4 or later | BD Multitest™ 4-Color |

a. To perform daily cytometer quality control.

b. To calculate compensation.

c. To set photomultiplier tube (PMT) voltages and fluorescence compensation, and check instrument sensitivity before use.

The BD FACS™ Loader and BD FACS™ Universal Loader can be used with this product. See the IFU for the cytometer used with your Loader for more information.

The BD FACSDuet™ sample preparation system can be used with this product. See the *BD FACSDuet™ Sample Preparation System Instructions for Use* for more information.

## 5. SPECIMEN COLLECTION AND PREPARATION

- Collect blood specimens aseptically by venipuncture into a BD Vacutainer® EDTA blood collection tube, or equivalent.[21]

  BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes has been validated with both liquid and dry formulations of EDTA. The reagent has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount™ Tubes.

  The assay requires 50 µL of peripheral blood per test. We recommend starting with a minimum of 100 µL of blood to accommodate the excess volume needed to perform reverse pipetting.
- If using the dual platform method, obtain a white blood cell (WBC) count and a differential white cell count from the same whole blood sample before staining to calculate absolute counts from percentages. See Dual Platform Method on page 15.
- Store blood specimens at room temperature (20–25 °C).
- Stain specimens within 48 hours of draw.
- Acquire samples within 24 hours of staining.

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[22,23] 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.[24]

### Interference

Substances present in the specimen might interfere with the assay:

- Specimens obtained from patients taking immunosuppressive drugs[25,26,27] or undergoing monoclonal antibody treatment[28,29,30,31,32,33] can yield erroneous results.
- Hemolyzed samples can interfere with the assay and should be rejected.[34] Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated before staining can give aberrant results.
- Blast cells can interfere with test results.[35]
- Lipemic specimens can interfere with the assay.[36,37]
- Bilirubin interferes at an absorbance peak of 456 nm.[38]

## Interfering Conditions

The following table lists the substances that were tested for interference with a similar reagent, the BD Multitest™ 6-Color TBNK reagent with optional BD Trucount™ Tubes.

Testing for interference was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[39] There was no detectable interference at the following concentrations.

**Table 3** Non-interfering substances

| Analyte | Concentration tested |
|---|---|
| Acetaminophen | 156 µg/mL |
| Acetylsalicylic acid (Aspirin) | 30 µg/mL |
| Albuterol | 0.015 µg/mL |
| Atenolol | 3 µg/mL |
| Atorvastatin | 0.25 µg/mL |
| Azithromycin | 3.7 µg/mL |
| Bilirubin, conjugated | 2 mg/dL |
| Cobicistat | 3.6 µg/mL |
| Efavirenz | 12 µg/mL |
| Enoxaparin | 2 µg/mL |
| Guaifenesin | 1.5 µg/mL |
| Hydroxychloroquine | 0.2 µg/mL |
| Ibuprofen | 73 µg/mL |
| Insulin | 37 µU/mL |
| Kaletra | 15.5 µg/mL |
| Lisinopril | 0.082 µg/mL |
| Maraviroc | 0.888 µg/mL |
| Oseltamivir | 0.133 µg/mL |
| Raltegravir | 15 µg/mL |
| Remdesivir | 16.32 µg/mL |
| Ritonavir | 15 µg/mL |
| Tenofovir | 0.978 µg/mL |
| Tocilizumab | 149.4 µg/mL |
| Vancomycin | 40 µg/mL |

The following substances interfered with the assay at the indicated concentration:

**Table 4** Interfering substances

| Analyte | Concentration tested |
|---|---|
| Albumin[a,e] | 6 g/dL |
| Bilirubin, unconjugated[b,e] | 2 mg/dL |
| Erythrocytes[c,e] | $6x10^3$ cells/µL |
| Hemoglobin[c,e] | 1000 mg/dL |
| Triglycerides[d,e] | 1500 mg/dL |

a. Albumin interferes as a result of its comparatively large concentration in the peripheral blood and its ability to bind as well as to release large quantities of ligands.40

b. Unconjugated Bilirubin may induce autofluorescence.41

c. The presence of red blood cells (RBCs) in the sample preparation can cause light interference and non-specific interactions leading to erroneous test results.42 Hemolyzed samples should be rejected. The hemoglobin concentration refers to free hemoglobin.

d. Immunomodulatory drugs used for treatment of HIV infection may cause lipemia. Lipemia is known to interfere in assays that use the transmission of light and impact the scattering of light.43,44

e. The listed endogenous substances interfere with the assay at higher than normal concentrations, i.e. hyperalbuminemia, unconjugated hyperbilirubinemia, erythrocytosis, hemoglobinemia, and hypertriglyceridemia. Interference caused by these endogenous substances is not uncommon and has been described in the literature (see references listed in notes a–d).

# 6. PROCEDURE

## Reagents and Materials

## Reagents and materials provided

BD Multitest™ CD3/CD8/CD45/CD4 is provided in 1 mL of buffered saline with <0.1% sodium azide. The reagent is sufficient for 50 tests.

If calculating absolute counts, use BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. The reagent comes with two pouches of BD Trucount™ Tubes. Each pouch contains 25 tubes, sufficient for 25 tests. The tubes contain a freeze-dried pellet of fluorescent beads in a single-use tube.

## Reagents and materials required but not provided

- BD FACS™ Lysing Solution (Catalog No. 349202)

  The lysing solution is provided as a 10X concentrate and it contains diethylene glycol and formaldehyde. See the *BD FACS™ Lysing Solution* IFU for precautions and warnings.
- Disposable 12 × 75-mm capped polystyrene test tubes, or equivalent (if not using BD Trucount™ Tubes)
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing 1X BD FACS™ Lysing Solution
- BD Multi-Check™ Control (Catalog Nos. 340911, 340912, 340913)
- BD Multi-Check™ CD4 Low Control (Catalog Nos. 340914, 340915, 340916)
- (Optional) BD Trucount™ Controls (Catalog No. 340335)
- (Optional) BD FACS™ Universal Loader
- (Optional) BD FACS™ Loader (used on the BD FACSCanto™ II flow cytometer)

## 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

Accurate pipetting is critical when using a BD Trucount™ Tube. Use the reverse pipetting technique to add the sample to a BD Trucount™ Tube. 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.

## Performing Quality Control

Run two levels of process control material (for example, BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control) before acquiring patient specimens.[45] Control materials should provide established values for percent positive and absolute counts for the relevant cell populations. Process the controls like patient specimens to monitor the performance of the entire analytic process. This is done at least once each day when patient testing is performed.

**NOTE** BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control are validated as process controls on BD FACSLyric™ flow cytometers.

If needed, use BD Trucount™ Controls to verify pipetting accuracy and the bead count value of the BD Trucount™ Tubes.

## Staining the Cells

If using the BD FACSDuet™ system to prepare the samples, see the *BD FACSDuet™ Sample Preparation System Instructions for Use*.

1. For each sample, remove a tube and label it with the appropriate sample identification.

   For calculating absolute counts and lymphocyte subset percentages, label a BD Trucount™ Tube. For calculating lymphocyte subset percentages only, label a 12 × 75-mm tube.

   **NOTE** For samples stained in BD Trucount™ Tubes, verify that the BD Trucount™ bead pellet is under 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. Do not transfer beads to another tube.
2. Pipette 20 µL of BD Multitest™ CD3/CD8/CD45/CD4 reagent into the bottom of the tube.

   If using a BD Trucount™ Tube, pipette the reagent onto the side of the tube, just above the metal retainer, without touching the bead pellet.
3. Pipette 50 µL of well-mixed control material or anticoagulated peripheral blood onto the side of the tube.

   If using a BD Trucount™ Tube, pipette the sample onto the side of the tube, just above the metal retainer, without touching the bead pellet.

   **NOTE** Thoroughly mix the controls before pipetting them. See the BD Multi-Check™ Control or BD Multi-Check™ CD4 Low Control IFU for detailed instructions.

   **NOTE** Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer. See Reverse Pipetting on page 7. Avoid smearing sample down the side of the tube. If whole blood or control material remains on the side of the tube, it will not be stained with the reagent and can affect results.
4. Cap the tube and vortex gently to mix.
5. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).
6. Add 450 µL of 1X BD FACS™ Lysing Solution to the tube.
7. Cap the tube and vortex gently to mix.
8. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).

   The sample is now ready to be analyzed on the flow cytometer. Acquire the sample within 24 hours of staining. Store the stained sample in the dark at room temperature (20–25 °C) until acquisition.

## Running the Assay on a BD FACSLyric™ Flow Cytometer

### Before you begin:

1. Ensure that Characterization QC (CQC) and lyse/no wash reference settings have not expired.
2. Add reagent lots to library, if needed.

   See the *BD FACSLyric™System Instructions For Use* for information.
3. Perform daily Performance QC (PQC) using BD® CS&T Beads.

   See the *BD® CS&T Beads* IFU and the *BD FACSLyric™ System Instructions For Use* for information.

### To run the assay:

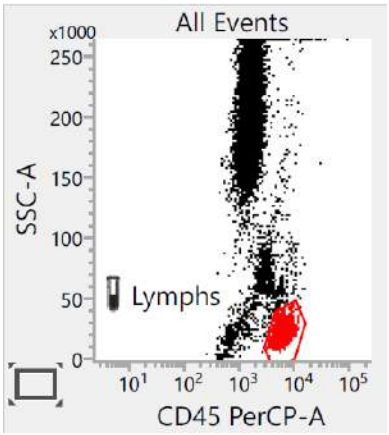
1. Create a worklist.
   - Create a Multi-Check™ Control task for each process control you are running.
   - Create an appropriate assay task for each patient specimen you are running.
2. Enter information in the worklist table.
   - If not using BD Trucount™ Tubes, enter the WBC count and the percentage of lymphocytes (WBC (x1000) and Lymphs (%), respectively), or the lymphocyte count (Lymphs (x1000)) in the appropriate column.

   **NOTE** Divide the WBC count or the lymphocyte count by 1,000 before entering it into the software.
   - If using BD Trucount™ Tubes, enter the lot ID for the tubes and the bead count, found on the pouch label, in the appropriate column (Trucount Lot ID and Beads Per Pellet, respectively).
3. Run the control tasks on the worklist.
4. Vortex each tube thoroughly at low speed immediately before acquiring it.[46]

   **NOTE** If you are using the BD FACS™ Universal Loader, vortex tubes immediately before placing them into the Loader racks.
5. After acquiring the control samples, click **Stop Tube**.

   **NOTE** This assumes that process control passes. Stop it to verify, then continue with samples of interest. If process control fails, restain samples and process controls because you cannot discriminate whether process control failure comes from staining or the instrument.
6. Review the lab report for the controls.
7. Visually inspect the CD45 PerCP-A vs SSC-A dot plot.

   The lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes should also appear as distinct clusters. Do not proceed with analysis if populations are diffuse and there is little or no separation between clusters.

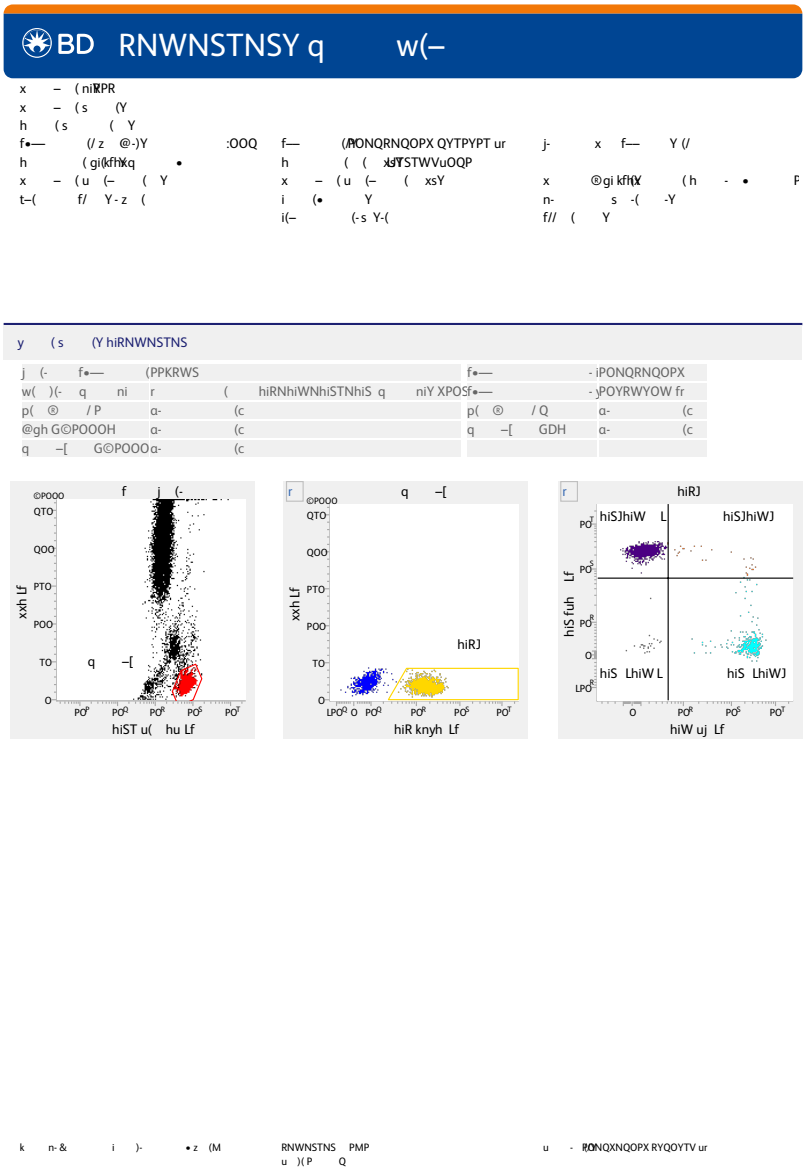



8. Verify that the results are within the values reported in the Assay Values sheet, provided with the controls.
9. Set the run pointer to the first patient specimen and select **Run from Pointer** from the **Run** menu.

Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.

10. Review the assay lab report.

    Page 1 of the lab report shows dot plots to identify the cell populations. The lab report shown is for BD Multitest™ CD3/CD8/CD45/CD4 without BD Trucount™ Tubes.



Page 2 of the lab report summarizes the results, presents QC results for the assay, and presents any QC messages that were triggered.

See the *BD FACSLyric™ System Instructions for Use* or the *BD FACSLyric™ Clinical Reference System* for more information.

## Running the Panel on a BD FACSCanto™ II Flow Cytometer

1. Run Setup using BD FACS™ 7-Color Setup Beads.
   See the *BD FACSCanto™ II Instructions for Use* for more information.
2. Add a BD Multitest™ CD3/CD8/CD45/CD4 panel entry for each process control and patient sample.

   **NOTE** The word "Control" must appear in the sample name of the process controls.

3. Acquire the process control samples.
4. Vortex each tube thoroughly at low speed immediately before acquiring it. It is important to reduce aggregation before running samples on the flow cytometer.

   **NOTE** If you are using the BD FACS™ Loader, vortex tubes immediately before placing them into the Loader racks.
5. Verify that the process control values are within the manufacturer's expected ranges.
6. Acquire the patient samples.

7. Review the assay lab report.

   The lab report shows dot plots to identify the cell populations, a table summarising the results, QC results, and any QC messages that were triggered. The lab report shown is for BD Multitest™ CD3/CD8/CD45/CD4 without BD Trucount™ Tubes.

**d7670ii**

Director:

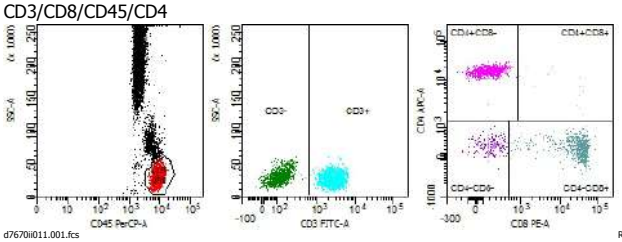
Panel: 3/8/45/4
Acquired: 10/28/2015 3:17:20 PM
Analyzed: 10/28/2015 3:17:20 PM
Status: OK
Operator: Lab Manager
Reviewer: 28102015.csv
Results:

WBC Count (x1000): | Lymphs (%): | Lymphs (x1000): 1.5

BD FACSCanto II V96300004 | BD FACSCanto v.3.1.5773.39345

CD3/CD8/CD45/CD4

Total Events: 10058




d7670ii011.001.fcs

Reagent Lot ID: 00000

| Parameter | Percent | Value/AbsCnt |
|---|---|---|
| Lymph Events | | 2600 |
| CD3+ | 61.15 | 917.31 |
| **CD3+CD8+ (Excl. dual pos.)** | **27.96** | **419.42** |
| **CD3+CD4+ (Excl. dual pos.)** | **26.23** | **393.46** |
| **CD3+CD4-CD8-** | **6.12** | **91.73** |
| CD3+CD4+CD8+ | 0.85 | 12.69 |
| CD3+CD8+ | 28.81 | 432.12 |
| CD3+CD4+ | 27.08 | 406.15 |
| CD45+ | | 1500 |
| **4/8 Ratio (Excl. dual pos.)** | | **0.94** |
| 4/8 Ratio | | 0.94 |

**QC Messages**
% T-Sum is: 5.27
4/8 ratio is: 0.94
**% T-Sum (Excl. dual pos.) is: 6.12**
**4/8 ratio (Excl. dual pos.) is: 0.94**

**Comments**

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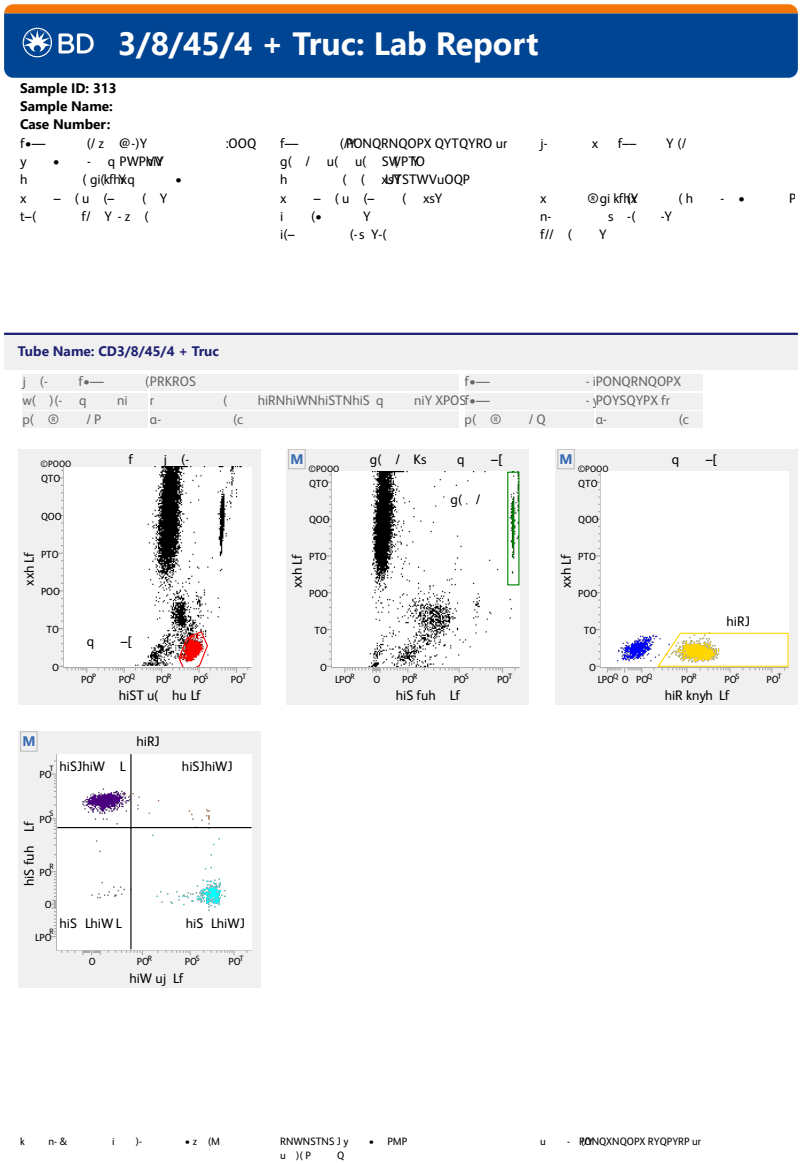
# 7. RESULTS

## Representative Data

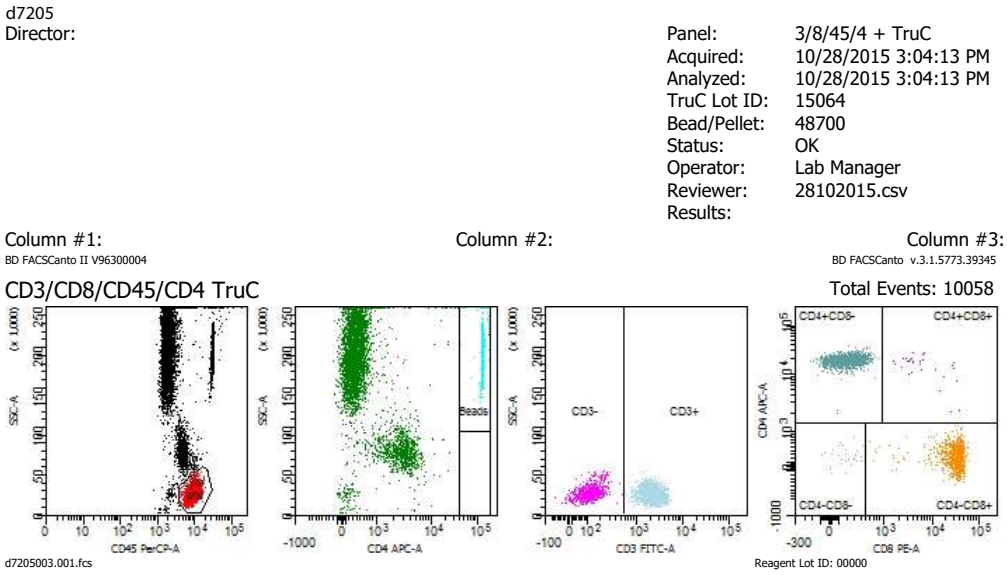
A hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube was acquired on a BD FACSLyric™ flow cytometer. See Figure 1.

**Figure 1** BD FACSLyric™ laboratory report showing data collected with BD Trucount™ Tubes.



A similar sample was acquired on a BD FACSCanto™ II flow cytometer.

**Figure 2** BD FACSCanto™ II laboratory report showing data collected with BD Trucount™ Tubes.




The lymphocyte subsets are identified using the following gating strategy:

**Table 5** Gating strategy for BD Multitest™ CD3/CD8/CD45/CD4

| Dot plot | Parent population | Gate | Populations identified |
|---|---|---|---|
| CD45 PerCP-A vs SSC-A | All Events | Lymphs | Lymphocytes |
| CD4 APC-A vs SSC-A | Beads, Not Lymphs | Beads | Trucount beads |
| CD3 FITC-A vs SSC-A | Lymphs | $CD3^+$ | $CD3^+$ T lymphocytes |
| CD8 PE-A vs CD4 APC-A | $CD3^+$ | Quadrant | $CD4^+CD8^-$<br>$CD4^+CD8^+$<br>$CD4^-CD8^+$<br>$CD4^-CD8^-$ |

The second dot plot, used to identify Trucount™ beads, is present in the 3/8/45/4 + Truc Lab Report only.

See the *BD FACSLyric™ Clinical Reference System*, which provides information on gating and troubleshooting.

## Calculating Absolute Counts

When using cytometer-specific BD software, results show positive cells as a percentage of lymphocytes. In addition, the software uses one of two methods to calculate absolute counts of positive cells per microliter of blood (cells/µL).

## Single Platform Method

When BD Trucount™ Tubes are used, the absolute number of positive cells in the sample can be determined by comparing cellular events to bead events. The software calculates absolute counts using the following formula:

$$\frac{\text{\# events in cell population}}{\text{\# events in absolute count bead region}} \times \frac{\text{\# beads/test}}{\text{test volume}} = \text{cell population absolute count}$$

The # beads/test is found on the BD Trucount™ Tubes foil pouch label and varies from lot to lot.

### Dual Platform Method

This method is used when using 12 × 75-mm polystyrene tubes (or equivalent) instead of BD Trucount™ Tubes. When creating the worklist, enter values for either the lymphocyte count, or the WBC count and the percentage of lymphocytes, as determined by a hematology analyzer or another method. See the instructions for use for your instrument for more information. The software uses one of the following formulas to calculate absolute counts:

- User provides lymphocyte count per µL.

$$\frac{\text{\# events in cell population} \times \text{lymphocyte count per µL}}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

- User provides WBC count per µL and percentage of lymphocytes.

$$\frac{\text{\# events in cell population} \times \text{WBC count} \times (\%\text{lymphocytes}/100)}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

**NOTE** The accuracy of the absolute counts determined with the Dual Platform Method depends upon the accuracy of the values entered into the software.

## 8. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the lymphocyte subsets identified using BD Multitest™ CD3/CD8/CD45/CD4. Age, gender, clinical characteristics, and ethnicity of patients should be known when a reference interval is determined.[47] The provided reference intervals are for information only.
- BD Multitest™ CD3/CD8/CD45/CD4 is not intended for screening samples for the presence of leukemic cells or for immunophenotyping samples from leukemia patients.
- Absolute counts are not comparable between laboratories using different manufacturers' equipment.
- BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants to determine absolute counts.

## 9. REFERENCE INTERVALS

Reference intervals for BD Multitest™ CD3/CD8/CD45/CD4 with and without BD Trucount™ Tubes were determined in a study using the BD FACSLyric™ flow cytometer.[41] The study objective was to establish device reference interval values in stained peripheral blood from a healthy cohort of male and female adults that are free of hematological abnormality. Device reference interval refers to a specified interval of the distribution of lymphocyte subset absolute count and percent values taken from a biological reference population. Blood from a population of healthy control subjects was stained with the BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes, and then acquired and analyzed on a BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application. See the first limitation (in the preceding section) for more information about reference intervals.

**Table 6** Representative reference intervals for BD Multitest™ CD3/CD8/CD45/CD4

| Lymphocyte subset | N[a] | Units | Mean | 95% range |
|---|---|---|---|---|
| $CD3^+$ | 130 | % | 72.00 | 56.65–83.36 |
| | | cells/µL | 1,551.28 | 840–2,641 |
| $CD3^+CD4^+$ | 130 | % | 46.51 | 32.42–63.19 |
| | | cells/µL | 1,003.50 | 488–1,711 |
| $CD3^+CD8^+$ | 130 | % | 23.25 | 8.99–38.99 |
| | | cells/µL | 514.19 | 154–1,097 |
| a. N = number of samples | | | | |

## 10. PERFORMANCE CHARACTERISTICS

### Specimen Handling and Collection (AOB/AOS)

A study was performed to assess the age of blood (AOB) and age of stain (AOS) using BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. The stability of EDTA-anticoagulated blood was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring stained sample

Whole blood specimens were tested to at least 51 hours post draw and stained samples were tested to at least 26 hours post stain. All samples were maintained at room temperature (20–25 °C) before staining or acquisition.

Based on the results of this study, we recommend staining samples within 48 hours of draw and analyzing samples within 24 hours of staining.

### Limit of blank and limit of detection

The detection capability of the BD Multitest™ CD3/CD8/CD45/CD4 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. Limit of Blank (LOB) refers to the highest apparent absolute count values that can be detected in a stained sample containing no lymphocytes. Limit of Detection (LOD) refers to the lowest absolute count values that can be detected above zero in a stained sample containing a very low $CD3^+CD4^+$ lymphocyte concentration.

Cell-free plasma samples were used to estimate LOB. Plasma samples containing 10 ±5 $CD3^+CD4^+$ cells/µL were used to estimate LOD. Sixty replicates of each sample type were stained manually or using the BD FACSDuet™ system with each of three reagent lots.

Three BD FACSLyric™ flow cytometers were used to acquire the manually prepared samples. A minimum of one BD FACSDuet™ system integrated with a BD FACSLyric™ flow cytometer was used in the other study. Absolute count values for LOB and LOD are shown in the following table.

**Table 7** Detection capability of BD Multitest™ CD3/CD8/CD45/CD4 (LOB and LOD)

| | Manual sample preparation | | Sample preparation with BD FACSDuet™ system | |
|---|---|---|---|---|
| **Lymphocyte subset** | **LOB (cells/µL)** | **LOD (cells/µL)** | **LOB (cells/µL)** | **LOD (cells/µL)** |
| $CD3^+$ | 4 | 9 | 2 | 8 |
| $CD3^+CD4^+$ | 4 | 8 | 2 | 6 |
| $CD3^+CD8^+$ | 4 | 11 | 2 | 6 |

## Limit of quantitation

The limit of quantitation (LOQ) of the BD Multitest™ CD3/CD8/CD45/CD4 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. LOQ refers to the lowest lymphocyte absolute count values that can be quantitatively detected with stated accuracy in samples containing a range of very low $CD3^+CD4^+$ concentration. Plasma samples containing 10, 20, 30, or 50 $CD3^+CD4^+$ cells/µL were used to estimate LOQ.

In the study on the BD FACSLyric™ flow cytometer, 40 replicates of samples from each of the four concentration levels were stained using two lots of the BD Multitest™ CD3/CD8/CD45/CD4 reagents. For the comparator system, 10 of the 40 replicates from each concentration level were stained and acquired on a BD FACSCanto™ II flow cytometer. Three BD FACSLyric™ flow cytometers and one BD FACSCanto™ II flow cytometer were used in the study.

In the study using the BD FACSDuet™ system, 10 replicates from each concentration level were stained with three lots of the reagents using the BD FACSDuet™ system and acquired using an integrated BD FACSLyric™ flow cytometer. For the comparator system, five replicates from each concentration level were stained manually with three lots of the reagents and acquired on a BD FACSLyric™ flow cytometer. Three integrated BD FACSDuet™–BD FACSLyric™ systems and one standalone BD FACSLyric™ flow cytometer were used in the study. Absolute count values for LOQ are shown in the following table.

**Table 8** Detection capability of BD Multitest™ CD3/CD8/CD45/CD4 (LOQ)

| | Manual sample preparation (first study) | Sample preparation with BD FACSDuet™ system (second study) |
|---|---|---|
| **Lymphocyte subset** | **LOQ (cells/µL)** | **LOQ (cells/µL)** |
| $CD3^+$ | 15 | 17 |
| $CD3^+CD4^+$ | 10 | 11 |
| $CD3^+CD8^+$ | 11 | 10 |

# BD FACSLyric™ Flow Cytometer

## Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer

A study was performed at five sites to demonstrate equivalency between acquisition using the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer. Peripheral blood specimens were collected from normal donors and HIV-infected individuals using BD Vacutainer® EDTA blood collection

tubes. Specimens were stained using BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and acquired on a BD FACSLyric™ flow cytometer using the BD FACSuite™ Clinical application. Lymphocyte subset percentages and absolute counts were enumerated. The results were compared with results from the same samples acquired on a BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software.

Method comparison statistics are reported for all cell subsets.[48] See the following table.

**Table 9** Method comparison statistics for lymphocyte subsets (BD FACSLyric™ flow cytometer)

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| $CD3^+$ | 362 | % | 0.99 | 1.00 | 0.68 | 1.29–98.35 |
| | | cells/µL | 0.99 | 1.03 | 3.18 | 6–9,197 |
| $CD3^+CD4^+$ | 362 | % | 1.00 | 1.01 | –0.22 | 0.12–97.72 |
| | | cells/µL | 1.00 | 1.03 | –0.05 | 1–7,739 |
| $CD3^+CD8^+$ | 362 | % | 1.00 | 1.00 | –0.08 | 0.22–82.93 |
| | | cells/µL | 0.99 | 1.02 | –1.35 | 1–5,774 |

## Method comparison, BD FACS™ Universal Loader vs manual acquisition

A single-site study was performed to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Peripheral blood specimens were stained in duplicate using BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. Stained samples were acquired on one of three BD FACSLyric™ flow cytometers using either the BD FACS™ Universal Loader or manual acquisition.

The mean, difference, and relative difference for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for lymphocyte subset percentages and absolute counts. See the following table.

**Table 10** BD FACS™ Universal Loader vs manual acquisition

| Lymphocyte subset | N | Units | Mean | | Difference | Relative difference |
|---|---|---|---|---|---|---|
| | | | Loader | Manual | | |
| $CD3^+$ | 72 | % | 74.09 | 73.93 | 0.16 | 0.28 |
| | | cells/µL | 1,504.39 | 1,501.17 | 3.22 | 0.48 |
| $CD3^+CD4^+$ | 72 | % | 28.46 | 28.55 | –0.09 | –0.78 |
| | | cells/µL | 567.62 | 572.35 | –4.72 | –0.41 |
| $CD3^+CD8^+$ | 72 | % | 43.04 | 42.92 | 0.12 | 0.46 |
| | | cells/µL | 887.74 | 882.47 | 5.26 | 0.60 |

## Method comparison, standalone BD FACSLyric™ vs BD FACSLyric™ with BD FACSDuet™ system

Peripheral blood specimens were collected at three clinical study sites. An aliquot of each specimen was stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube using the BD FACSDuet™ system. Stained samples were automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application. A second aliquot of each specimen was stained manually with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube. Stained samples were acquired on a standalone BD FACSLyric™ flow cytometer using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application.

Results were compared between samples prepared using the BD FACSDuet™ system and samples prepared manually. See the following table.

**Table 11** Method comparison statistics for lymphocyte subsets

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| $CD3^+$ | 373 | % | 0.98 | 0.99 | 0.54 | 45.3–99.21 |
| | | cells/µL | 0.98 | 1.00 | 5.73 | 93–11,138 |
| $CD3^+CD4^+$ | 373 | % | 0.99 | 1.00 | –0.02 | 0.37–91.86 |
| | | cells/µL | 0.99 | 1.00 | –0.15 | 4–7,911 |
| $CD3^+CD8^+$ | 373 | % | 0.99 | 0.99 | –0.01 | 2.52–86.68 |
| | | cells/µL | 0.98 | 0.99 | 3.16 | 52–5,796 |

## Precision (repeatability), control material (standalone BD FACSLyric™ flow cytometer)

A 21-day single-site precision study was performed to assess repeatability and within-site precision using control material.[49] Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across four BD FACSLyric™ flow cytometers and four operators by acquiring two concentrations of analyte, CD-Chex Plus® control (CDN) and CD-Chex Plus® CD4 Low control (CDL), stained in duplicate using four lots of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts using control material, respectively.

**Table 12** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 76.81 | 0.80 | 0.83 |
| $CD3^+CD4^+$ | 50.74 | 1.01 | 1.02 |
| $CD3^+CD8^+$ | 22.22 | 0.80 | 0.80 |

**Table 13** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 57.31 | 1.13 | 1.18 |
| $CD3^+CD4^+$ | 11.66 | 0.62 | 0.64 |
| $CD3^+CD8^+$ | 40.36 | 1.04 | 1.06 |

**Table 14** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,729.61 | 3.85 | 4.03 |
| $CD3^+CD4^+$ | 1,142.52 | 4.04 | 4.18 |
| $CD3^+CD8^+$ | 500.42 | 5.56 | 5.67 |

**Table 15** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 869.06 | 4.24 | 4.32 |
| $CD3^+CD4^+$ | 176.91 | 6.59 | 6.67 |
| $CD3^+CD8^+$ | 612.12 | 4.55 | 4.65 |

## Precision (repeatability), control material (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A 21-day single-site precision study was performed to assess repeatability and within-site precision when samples were prepared and acquired on the BD FACSLyric™ flow cytometer with BD FACSDuet™ sample preparation system using control material. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three BD FACSDuet™ systems, each integrated with a BD FACSLyric™ flow cytometer, and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), stained in duplicate using three lots of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for within-site precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

**Table 16** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 77.43 | 0.88 | 0.88 |
| $CD3^+CD4^+$ | 48.79 | 0.96 | 0.96 |
| $CD3^+CD8^+$ | 26.77 | 0.91 | 0.91 |

**Table 17** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 63.69 | 1.02 | 1.03 |
| $CD3^+CD4^+$ | 14.94 | 0.68 | 0.70 |
| $CD3^+CD8^+$ | 44.04 | 1.11 | 1.12 |

**Table 18** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,751.48 | 5.10 | 6.86 |
| $CD3^+CD4^+$ | 1,103.49 | 5.23 | 6.88 |
| $CD3^+CD8^+$ | 605.62 | 6.23 | 7.70 |

**Table 19** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 738.14 | 4.06 | 4.92 |
| $CD3^+CD4^+$ | 173.18 | 5.97 | 6.57 |
| $CD3^+CD8^+$ | 510.44 | 4.40 | 5.28 |

## Precision (repeatability), peripheral blood (standalone BD FACSLyric™ flow cytometer)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 44 donor samples. Each donor sample was stained in duplicate using BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and run on 12 instruments for a total of 24 runs per sample.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts using peripheral blood, respectively.

**Table 20** Repeatability and within-site precision of lymphocyte subset percentages

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 73.54 | 0.96 | 0.96 |
| $CD3^+CD4^+$ | 33.46 | 0.83 | 0.83 |
| $CD3^+CD8^+$ | 37.93 | 0.93 | 0.93 |

**Table 21** Repeatability and within-site precision of lymphocyte subset absolute counts

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,400.10 | 4.49 | 4.61 |
| $CD3^+CD4^+$ | 633.59 | 5.32 | 5.40 |
| $CD3^+CD8^+$ | 726.59 | 5.42 | 5.53 |

## Precision (repeatability), peripheral blood (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 27 donor specimens. Each donor specimen was stained in duplicate using three lots of BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and run on three BD FACSDuet™ instruments, each integrated with a BD FACSLyric™ flow cytometer, for a total of 18 runs per sample.

**Table 22** Repeatability and within-site precision of lymphocyte subset percentages

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 76.62 | 0.91 | 0.91 |
| $CD3^+CD4^+$ | 31.18 | 0.89 | 0.89 |
| $CD3^+CD8^+$ | 44.04 | 1.01 | 1.05 |

**Table 23** Repeatability and within-site precision of lymphocyte subset absolute counts

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,566.95 | 4.13 | 4.43 |
| $CD3^+CD4^+$ | 636.55 | 5.12 | 5.38 |
| $CD3^+CD8^+$ | 905.37 | 5.17 | 5.65 |

## Precision (reproducibility), control material (standalone BD FACSLyric™ flow cytometer)

A study was performed at four clinical sites to assess reproducibility of BD Multitest™ CD3/CD8/CD45/CD4. A single lot of each process control, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), was provided to each site. The control samples were stained using BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of five non-consecutive tested days for a total of 10 runs.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for reproducibility of lymphocyte subset percentages and absolute counts, respectively.

**Table 24** Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 76.64 | 0.91 |
| $CD3^+CD4^+$ | 51.67 | 1.58 |
| $CD3^+CD8^+$ | 23.23 | 0.85 |

**Table 25** Reproducibility of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 57.14 | 1.21 |
| $CD3^+CD4^+$ | 12.12 | 0.61 |
| $CD3^+CD8^+$ | 40.74 | 1.12 |

**Table 26** Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 1,746.97 | 4.65 |
| $CD3^+CD4^+$ | 1,177.59 | 5.17 |
| $CD3^+CD8^+$ | 529.63 | 6.05 |

**Table 27** Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 881.62 | 5.03 |
| $CD3^+CD4^+$ | 187.01 | 7.30 |
| $CD3^+CD8^+$ | 628.51 | 5.23 |

## Precision (reproducibility), control material (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A study was performed at three clinical sites to assess reproducibility of BD Multitest™ CD3/CD8/CD45/CD4. A single lot of each process control, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), was provided to each site. The control samples were stained using three lots of BD Multitest™ CD3/CD8/CD45/CD4 with one lot of BD Trucount™ Tubes using the BD FACSDuet™ system and automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using the BD FACS™ Universal Loader. Two separate runs were performed each day. Results obtained over 15 non-consecutive test days were analyzed.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for reproducibility of lymphocyte subset percentages and absolute counts, respectively.

**Table 28** Reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 75.99 | 0.85 |
| $CD3^+CD4^+$ | 49.83 | 0.91 |
| $CD3^+CD8^+$ | 24.60 | 0.73 |

**Table 29** Reproducibility of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 57.15 | 1.02 |
| $CD3^+CD4^+$ | 9.97 | 0.61 |
| $CD3^+CD8^+$ | 42.94 | 1.05 |

**Table 30** Reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 1,987.36 | 6.39 |
| $CD3^+CD4^+$ | 1,303.23 | 6.62 |
| $CD3^+CD8^+$ | 643.31 | 7.28 |

**Table 31** Reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 957.91 | 6.32 |
| $CD3^+CD4^+$ | 167.16 | 8.47 |
| $CD3^+CD8^+$ | 719.77 | 6.68 |

## Linearity (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

Linearity was assessed for the BD FACSLyric™ flow cytometer, with and without an integrated BD FACSDuet™ system, using triplicate measurements of 11 equally spaced concentrations of WBCs. Lymphocyte subsets were observed to be linear across the following ranges.

**Table 32** Linear ranges of lymphocyte subsets

| | Range (cells/µL) | |
|---|---|---|
| **Lyphocyte subset** | **BD FACSLyric™** | **BD FACSLyric™ with BD FACSDuet™** |
| $CD3^+$ | 3–5,148 | 4–5,318 |
| $CD3^+CD4^+$ | 1–3,184 | 3–3,016 |
| $CD3^+CD8^+$ | 7–3,480 | 2–3,130 |

## Measuring range (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

The analytical measurement range (AMR) for BD Multitest™ CD3/CD8/CD45/CD4 on the BD FACSLyric™ flow cytometer was determined. To establish the measuring range of the BD Multitest™ CD3/CD8/CD45/CD4, data was taken from the following:

- The LOQ studies using the BD FACSLyric™ flow cytometer with and without the BD FACSDuet™ system.
- The method comparison study between the BD FACSLyric™ and the BD FACSCanto™ II flow cytometers.
- The method comparison study between the standalone BD FACSLyric™ flow cytometer and the BD FACSLyric™ with BD FACSDuet™ system.

The lower end of the AMR was determined based on results from the limit of quantitation (LoQ) studies and the upper end of the AMR was determined based on results from the method comparison studies.

**Table 33** BD Multitest™ CD3/CD8/CD45/CD4 measuring range

| Lymphocyte subset | Analytical measuring range (cells/µL) |
|---|---|
| $CD3^+$ | 17–5,000 |
| $CD3^+CD4^+$ | 11–3,000 |
| $CD3^+CD8^+$ | 11–3,000 |

# BD FACSCanto™ II Flow Cytometer

## Method comparison, BD FACSCanto™ II vs BD FACSCanto™ flow cytometer

Lymphocyte subset percentages and absolute counts were enumerated with BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and analyzed on the BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software v2.1. The results were compared with results from the same samples analyzed on the BD FACSCanto™ flow cytometer using BD FACSCanto™ Clinical Software v2.0.

Peripheral blood samples were collected at random at two clinical laboratories. Method comparison statistics are reported in the following table.

**Table 34** Method comparison statistics for subset percentages and absolute counts (BD FACSCanto™ II vs BD FACSCanto™ flow cytometer)

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| Average $CD3^+$ | 104 | % | 0.984 | 0.97 | 2.72 | 51–92 |
| | | cells/µL | 0.991 | 0.97 | 27.59 | 221–3,872 |
| $CD3^+CD4^+$ | 104 | % | 0.994 | 1.01 | 0.20 | 1–57 |
| | | cells/µL | 0.986 | 0.95 | 18.25 | 11–1,905 |
| $CD3^+CD8^+$ | 104 | % | 0.993 | 1.00 | 0.34 | 11–81 |
| | | cells/µL | 0.988 | 0.95 | 28.36 | 68–3,577 |

## Precision (repeatability), control material (BD FACSCanto™ II flow cytometer)

A 21-day single-site study was conducted assess repeatability precision. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three instruments and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low (CDL) control, stained in duplicate using one lot of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables provide SDs and %CVs for subset percentages and absolute counts for repeatability and within-site precision.

**Table 35** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| Average $CD3^+$ | 73.0 | 0.63 | 0.67 |
| $CD3^+CD4^+$ | 46.8 | 0.81 | 0.82 |
| $CD3^+CD8^+$ | 25.4 | 0.78 | 0.80 |

**Table 36** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| Average $CD3^+$ | 54.1 | 0.96 | 0.98 |
| $CD3^+CD4^+$ | 10.3 | 0.53 | 0.53 |
| $CD3^+CD8^+$ | 43.2 | 1.33 | 1.34 |

**Table 37** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| Average $CD3^+$ | 2,105.4 | 2.7 | 2.9 |
| $CD3^+CD4^+$ | 1,347.1 | 3.6 | 3.8 |
| $CD3^+CD8^+$ | 731.4 | 4.5 | 4.7 |

**Table 38** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| Average $CD3^+$ | 1,086.0 | 3.5 | 3.6 |
| $CD3^+CD4^+$ | 205.6 | 5.9 | 5.9 |
| $CD3^+CD8^+$ | 866.1 | 3.8 | 3.9 |

## Linearity (BD FACSCanto™ II flow cytometer)

Linearity of the BD Multitest™ CD3/CD8/CD45/CD4 assay using BD Trucount™ Tubes was assessed for the BD FACSCanto™ II system within a WBC concentration of 0 to $3.8 \times 10^4$ cells/µL. Results were observed to be linear across the following range.

**Table 39** Linear ranges of lymphocyte subsets

| Lymphocyte subset | Range (cells/µL) |
|---|---|
| Average $CD3^+$ | 4–5,998 |
| $CD3^+CD4^+$ | 1–3,669 |
| $CD3^+CD8^+$ | 2–2,324 |

# 11. TROUBLESHOOTING

| Problem | Possible Cause | Solution |
|---|---|---|
| Poor resolution between debris and lymphocytes. | Cell interaction with other cells and platelets. | Prepare and stain another sample. |
| | Rough handling during cell preparation. | Check cell viability. Centrifuge cells at lower speed. |
| | Inappropriate instrument settings. | Follow proper instrument setup procedures. Optimize instrument settings as required. |

| Problem | Possible Cause | Solution |
|---|---|---|
| Staining dim or fading. | Cell concentration too high at staining step. | Check and adjust cell concentration or sample volume. Stain with fresh sample. |
| | Insufficient reagent. | Repeat staining with increased amount of antibody. |
| | Cells not analyzed within 24 hours of staining. | Repeat staining with fresh sample. Analyze promptly. |
| Few or no cells. | Cell concentration too low. | Resuspend fresh sample at a higher concentration. Repeat staining and analysis. |
| | Cytometer malfunctioning. | Troubleshoot instrument. |

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## NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

## 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.



## HISTORY

| Revision | Date | Changes made |
|---|---|---|
| 23-5351(11) | 2022-12 | Updated to meet requirements of Regulation (EU) 2017/746. |
| 23-5351(12) | 2023-07 | Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary. |

**Symbols Glossary**

Please refer to product labeling for applicable symbols.

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|  | Authorized representative in the European Community |
|  | Authorised representative in Switzerland |
|  | Date of manufacture |
|  | Use-by date |
|  | Batch code |
|  | Catalogue number |
|  | Serial number |
|  | Sterile |
|  | Sterilized using aseptic processing techniques |
|  | Sterilized using ethylene oxide |
|  | Sterilized using irradiation |
|  | Sterilized using steam or dry heat |
|  | Do not resterilize |
|  | Non-sterile |
|  | Do not use if package is damaged and consult *instructions for use* |
|  | Sterile fluid path |
|  | Sterile fluid path (ethylene oxide) |
|  | 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 |
|  | Contains or presence of natural rubber latex |
|  | In vitro diagnostic medical device |
|  | Negative 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 |
|  | Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP) |
|  | Collect separately<br>Indicates separate collection for waste of electrical and electronic equipment required. |
|  | CE marking; Signifies European technical conformity |
|  | Device for near-patient testing |
|  | Device for self-testing |
|  | This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner." |
|  | Country of manufacture<br>"CC" shall be replaced by either the two letter or the three letter country code. |
|  | Collection time |
|  | Cut |
|  | Peel here |
|  | Collection date |
|  | Keep away from light |
|  | Hydrogen gas is generated |
|  | Perforation |
|  | Start panel sequence number |
|  | End panel sequence number |
|  | Internal sequence number |
|  | <Box #> / <Total Boxes> |
|  | Medical device |
|  | Contains hazardous substances |
|  | Ukrainian conformity mark |
|  | Meets FCC requirements per 21 CFR Part 15 |
|  | UL product certification for US and Canada |
|  | Unique device identifier |
|  | Importer |
|  | Place patient label in framed area only |
|  | Magnetic resonance (MR) safe |
|  | Magnetic resonance (MR) conditional |
|  | Magnetic resonance (MR) unsafe |
|  | For use with |
|  | This Product Contains Dry Natural Rubber |
|  | For Export Only |
|  | Instruments |

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

L006715(08) 2023-03

## CONTACT INFORMATION

**Becton, Dickinson and Company**
**BD Biosciences**
155 North McCarthy Boulevard
Milpitas, California 95035 USA

EC REP **Becton Dickinson Ireland Ltd.**
Donore Road, Drogheda
Co. Louth, A92 YW26
Ireland

**Becton Dickinson Distribution Center NV**
Laagstraat 57
9140 Temse, Belgium

CH REP **BD Switzerland Sàrl**
Route de Crassier 17
Business Park Terre-Bonne
Bâtiment A4
1262 Eysins
Switzerland

**Becton Dickinson AG**
Binningerstrasse 94
4123 Allschwil
Switzerland

**BD Biosciences**
**European Customer Support**
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand Distributors:

**Becton Dickinson Pty Ltd.**
66 Waterloo Road
Macquarie Park NSW 2113
Australia

**Becton Dickinson Limited**
14B George Bourke Drive
Mt. Wellington Auckland 1060
New Zealand

Technical Service and Support: In the United States contact BD at 1.877.232.8995 or bdbiosciences.com.
For regions outside the United States, contact your local BD representative or bdbiosciences.com.

ClinicalApplications@bd.com

eifu.bd.com

**Becton, Dickinson and Company**
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

# SAFETY DATA SHEET

## 1. Identification

**Product identifier**

| Product No.: | Product name: | Common name(s), synonym(s) |
|---|---|---|
| 340345 | BD® FACSClean | No data available |

**Other means of identification**
**SDS number:** 088100018880

**Recommended use and restriction on use**

**Recommended use:** Scientific and Industrial laboratory use.
**Restrictions on use:** None known.

**Manufacturer/Importer/Supplier/Distributor Information**

**Manufacturer**

| | |
|---|---|
| Company Name: | Becton, Dickinson and Company - BD Biosciences |
| Address: | 2350 Qume Drive<br>95131 San Jose, CA USA |
| Telephone: | 1 877 232 8995 or 1 800 424 9300 |
| Fax: | |
| Contact Person: | Technical Services |
| E-mail: | ResearchApplications@bd.com or ClinicalApplications@bd.com |

**Emergency telephone number:** CHEMTREC 1 800 424 9300

## 2. Hazard(s) identification

**Hazard Classification**

**Health Hazards**

| | |
|---|---|
| Skin Corrosion/Irritation | Category 2 |
| Serious Eye Damage/Eye Irritation | Category 2A |

**Environmental Hazards**

| | |
|---|---|
| Acute hazards to the aquatic environment | Category 2 |
| Chronic hazards to the aquatic environment | Category 3 |

**Label Elements**

**Hazard Symbol:**

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**Signal Word:** Warning

**Hazard Statement:**
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H401: Toxic to aquatic life.
H412: Harmful to aquatic life with long lasting effects.

**Precautionary Statements**

**Prevention:**
P264: Wash thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P273: Avoid release to the environment.

**Response:**
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313: If eye irritation persists: Get medical advice/attention.
P302+P352: IF ON SKIN: Wash with plenty of water/…
P332+P313: If skin irritation occurs: Get medical advice/attention.
P321: Specific treatment (see on this label).
P362: Take off contaminated clothing.

**Disposal:**
P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

**Other hazards which do not result in GHS classification:** None.

## 3. Composition/information on ingredients

**Mixtures**

| Chemical Identity | Common name and synonyms | CAS number | Content in percent (%)* |
|---|---|---|---|
| Hypochlorous acid, sodium salt (1:1) | No data available. | 7681-52-9 | 1% |
| Sodium hydroxide (Na(OH)) | No data available. | 1310-73-2 | 0.8% |

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

## 4. First-aid measures

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| | |
|---|---|
| **General information:** | Causes serious eye irritation. Causes skin irritation. |
| **Ingestion:** | DO NOT induce vomiting. Get medical attention immediately. |
| **Inhalation:** | Provide fresh air, warmth and rest, preferably in comfortable upright sitting position. |
| **Skin Contact:** | Promptly flush contaminated skin with soap or mild detergent and water. Promptly remove clothing if penetrated and flush the skin with water. |
| **Eye contact:** | Immediately flush with plenty of water for at least 15 minutes. If easy to do, remove contact lenses. Get medical attention. |

**Most important symptoms/effects, acute and delayed**

| | |
|---|---|
| **Symptoms:** | No data available. |
| **Hazards:** | Causes serious eye irritation. Causes skin irritation. |

**Indication of immediate medical attention and special treatment needed**

| | |
|---|---|
| **Treatment:** | Get medical attention if symptoms occur. |

## 5. Fire-fighting measures

| | |
|---|---|
| **General Fire Hazards:** | Extinguish all ignition sources. Avoid sparks, flames, heat and smoking. Ventilate. Use water to keep fire exposed containers cool and disperse vapors. |

**Suitable (and unsuitable) extinguishing media**

| | |
|---|---|
| **Suitable extinguishing media:** | Use fire-extinguishing media appropriate for surrounding materials. |
| **Unsuitable extinguishing media:** | Avoid water in straight hose stream; will scatter and spread fire. |
| **Specific hazards arising from the chemical:** | Fire or excessive heat may produce hazardous decomposition products. |

**Special protective equipment and precautions for firefighters**

| | |
|---|---|
| **Special fire fighting procedures:** | No unusual fire or explosion hazards noted. |
| **Special protective equipment for fire-fighters:** | Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA. |

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## 6. Accidental release measures

**Personal precautions, protective equipment and emergency procedures:** Contact local authorities in case of spillage to drain/aquatic environment. Ensure suitable personal protection (including respiratory protection) during removal of spillages in a confined area.

**Methods and material for containment and cleaning up:** Absorb spillage with suitable absorbent material. Prevent runoff from entering drains, sewers, or streams. See Section 8 of the SDS for Personal Protective Equipment. For waste disposal, see section 13 of the SDS.

**Environmental Precautions:** Avoid release to the environment.

## 7. Handling and storage

**Precautions for safe handling:** When using do not eat, drink or smoke. Read and follow manufacturer's recommendations. Use personal protective equipment as required.

**Conditions for safe storage, including any incompatibilities:** Store in a cool, dry place. Keep container tightly closed. Keep from contact with oxidizing materials.

## 8. Exposure controls/personal protection

### Control Parameters

#### Occupational Exposure Limits

| Chemical Identity | Type | Exposure Limit Values | Source |
|---|---|---|---|
| Sodium hydroxide (Na(OH)) | Ceiling | 2 mg/m3 | US. OSHA Table Z-1-A (29 CFR 1910.1000), as amended (1989) |
| | Ceiling | 2 mg/m3 | US. Tennessee. OELs. Occupational Exposure Limits, Table Z1A, as amended (06 2008) |
| Sodium hydroxide (Na(OH)) - Particulate. | AN ESL | 2 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality), as amended (07 2011) |
| | ST ESL | 20 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality), as amended (07 2011) |
| Sodium hydroxide (Na(OH)) | Ceiling | 2 mg/m3 | US. California Code of Regulations, Title 8, Section 5155. Airborne Contaminants, as amended (08 2010) |
| | Ceiling | 2 mg/m3 | US. ACGIH Threshold Limit Values, as amended (12 2010) |
| | Ceil_Time | 2 mg/m3 | US. NIOSH: Pocket Guide to Chemical Hazards, as amended (2005) |
| | PEL | 2 mg/m3 | US. OSHA Table Z-1 Limits for Air Contaminants (29 CFR 1910.1000), as amended (02 2006) |
| | IDLH | 10 mg/m3 | US. NIOSH. Immediately Dangerous to Life or Health (IDLH) Values, as amended (10 2017) |

**Appropriate Engineering Controls** No special requirements under ordinary conditions of use and with adequate ventilation.

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**Individual protection measures, such as personal protective equipment**

| | |
|---|---|
| **General information:** | Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants. |
| **Eye/face protection:** | Wear safety glasses with side shields (or goggles). |
| **Skin Protection** | |
| **Hand Protection:** | Chemical resistant gloves Suitable gloves can be recommended by the glove supplier. Wash hands after contact. |
| **Other:** | Wear a lab coat or similar protective clothing. |
| **Respiratory Protection:** | If engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn. |
| **Hygiene measures:** | Observe good industrial hygiene practices. |

## 9. Physical and chemical properties

**Appearance**

| | |
|---|---|
| **Physical state:** | liquid |
| **Form:** | Aqueous Solution |
| **Color:** | Colorless |
| **Odor:** | Characteristic |
| **Odor threshold:** | No data available. |
| **pH:** | No data available. |
| **Melting point/freezing point:** | No data available. |
| **Initial boiling point and boiling range:** | No data available. |
| **Flash Point:** | No data available. |
| **Evaporation rate:** | No data available. |
| **Flammability (solid, gas):** | No data available. |
| **Upper/lower limit on flammability or explosive limits** | |
| **Flammability limit - upper (%):** | No data available. |
| **Flammability limit - lower (%):** | No data available. |
| **Explosive limit - upper (%):** | No data available. |
| **Explosive limit - lower (%):** | No data available. |
| **Vapor pressure:** | No data available. |
| **Vapor density:** | No data available. |
| **Relative density:** | No data available. |
| **Solubility(ies)** | |
| **Solubility in water:** | No data available. |
| **Solubility (other):** | No data available. |

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| | |
|---|---|
| **Partition coefficient (n-octanol/water):** | No data available. |
| **Auto-ignition temperature:** | No data available. |
| **Decomposition temperature:** | No data available. |
| **Viscosity:** | No data available. |

## 10. Stability and reactivity

| | |
|---|---|
| **Reactivity:** | Product is not reactive under normal conditions and recommended use. |
| **Chemical Stability:** | Material is stable under normal conditions. |
| **Possibility of hazardous reactions:** | Material is stable under normal conditions. |
| **Conditions to avoid:** | Avoid exposure to high temperatures or direct sunlight. |
| **Incompatible Materials:** | Water reactive material. Metals. Avoid contact with oxidizers or reducing agents. Avoid contact with acids. |
| **Hazardous Decomposition Products:** | Contact with acids liberates toxic gas. Stable; however, may decompose if heated. |

## 11. Toxicological information

**Information on likely routes of exposure**

| | |
|---|---|
| **Ingestion:** | No data available. |
| **Inhalation:** | No data available. |
| **Skin Contact:** | No data available. |
| **Eye contact:** | No data available. |

**Symptoms related to the physical, chemical and toxicological characteristics**

| | |
|---|---|
| **Ingestion:** | No data available. |
| **Inhalation:** | No data available. |
| **Skin Contact:** | No data available. |
| **Eye contact:** | No data available. |

**Information on toxicological effects**

**Acute toxicity (list all possible routes of exposure)**

**Oral**

| | |
|---|---|
| **Product:** | No data available. |

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**Dermal**

| | |
|---|---|
| **Product:** | No data available. |

**Inhalation**

| | |
|---|---|
| **Product:** | ATEmix: 525 mg/l |

**Repeated dose toxicity**

| | |
|---|---|
| **Product:** | No data available. |

**Specified substance(s):**

| | |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | LOAEL (Rat(Female), Oral, 90 d): > 24.9 mg/kg Oral Experimental result, Key study<br>LOAEL (Mouse(Female, Male), Oral, 90 d): > 34.4 mg/kg Oral Experimental result, Key study<br>LOAEL (Rat(Female, Male), Inhalation): <= 3 mg/m3 Inhalation Read-across from supporting substance (structural analogue or surrogate), Supporting study<br>LOAEL (Rat(Male), Oral, 90 d): > 16.7 mg/kg Oral Experimental result, Key study<br>NOAEL (Rat(Female), Oral, 90 d): >= 24.9 mg/kg Oral Experimental result, Key study |

**Skin Corrosion/Irritation**

| | |
|---|---|
| **Product:** | No data available. |

**Specified substance(s):**

| | |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | in vivo (Rabbit): Irritating Experimental result, Supporting study |
| Sodium hydroxide (Na(OH)) | in vivo (Rabbit): Irritating Experimental result, Weight of Evidence study<br>in vivo (Rabbit): Slightly irritating Experimental result, Weight of Evidence study |

**Serious Eye Damage/Eye Irritation**

| | |
|---|---|
| **Product:** | No data available. |

**Specified substance(s):**

| | |
|---|---|
| Sodium hydroxide (Na(OH)) | in vivo (Rabbit, 1 d): Mild irritant OECD GHS<br>in vivo (Rabbit, 2 d): Mild irritant OECD GHS<br>in vivo (Rabbit, 3 d): Mild irritant OECD GHS<br>in vivo (Rabbit, 4 d): Mild irritant OECD GHS |

**Respiratory or Skin Sensitization**

| | |
|---|---|
| **Product:** | No data available. |

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**Specified substance(s):**

| | |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | Skin sensitization:, in vivo (Guinea pig): Non sensitising |

**Carcinogenicity**

**Product:** No data available.

**IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:**

No carcinogenic components identified

**US. National Toxicology Program (NTP) Report on Carcinogens:**

No carcinogenic components identified

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050), as amended:**

No carcinogenic components identified

**Germ Cell Mutagenicity**

**In vitro**

**Product:** No data available.

**In vivo**

**Product:** No data available.

**Reproductive toxicity**

**Product:** No data available.

**Specific Target Organ Toxicity - Single Exposure**

**Product:** No data available.

**Specific Target Organ Toxicity - Repeated Exposure**

**Product:** No data available.

**Aspiration Hazard**

**Product:** No data available.

**Other effects:** No data available.

## 12. Ecological information

**Ecotoxicity:**

**Acute hazards to the aquatic environment:**

**Fish**

**Product:** Toxic to aquatic organisms.

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**Aquatic Invertebrates**
**Product:** Toxic to aquatic organisms.

**Chronic hazards to the aquatic environment:**

**Fish**
**Product:** Substantial amounts of the product may lead to a local change in acidity in small water systems which may have adverse effects on aquatic organisms.

**Aquatic Invertebrates**
**Product:** Aquatic plants and animals may be adversely affected if they have direct contact with this material.

**Toxicity to Aquatic Plants**
**Product:** No data available.

**Persistence and Degradability**

**Biodegradation**
**Product:** The subject product is expected to biodegrade and is not expected to persist for long periods in an aquatic environment.

**BOD/COD Ratio**
**Product:** No data available.

**Bioaccumulative potential**
**Bioconcentration Factor (BCF)**
**Product:** No data available.

**Partition Coefficient n-octanol / water (log Kow)**
**Product:** No data available.

**Mobility in soil:** No data available.

**Known or predicted distribution to environmental compartments**

| | |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | No data available. |
| Sodium hydroxide (Na(OH)) | No data available. |

**Other adverse effects:** None known.

## 13. Disposal considerations

**General information:** This material and its container must be disposed of as hazardous waste. Dispose of waste and residues in accordance with local authority requirements.

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**Disposal instructions:** Dispose of waste at an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

**Contaminated Packaging:** No data available.

## 14. Transport information

| | |
|---|---|
| **DOT**UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Label(s): | Not regulated. |
| Packing Group: | Not regulated. |
| Marine Pollutant: | Not regulated. |
| Limited quantity | Not regulated. |
| Excepted quantity | Not regulated. |
| Special precautions for user: | Not regulated. |

**IMDG**

| | |
|---|---|
| UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| EmS No.: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine Pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

**IATA**

| | |
|---|---|
| UN Number: | Not regulated. |
| Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es): | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

## 15. Regulatory information

**US Federal Regulations**

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**TSCA Section 12(b) Export Notification (40 CFR 707, Subpt. D)**
None present or none present in regulated quantities.

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050), as amended**
None present or none present in regulated quantities.

**CERCLA Hazardous Substance List (40 CFR 302.4):**

| Chemical Identity | Reportable quantity |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | 100 lbs. |
| Sodium hydroxide (Na(OH)) | 1000 lbs. |

**Superfund Amendments and Reauthorization Act of 1986 (SARA)**

**Hazard categories**
Immediate (Acute) Health Hazards
Skin Corrosion or Irritation
Serious eye damage or eye irritation

**SARA 302 Extremely Hazardous Substance**
None present or none present in regulated quantities.

**SARA 304 Emergency Release Notification**
None present or none present in regulated quantities.

**SARA 311/312 Hazardous Chemical**

| Chemical Identity | Threshold Planning Quantity |
|---|---|

**SARA 313 (TRI Reporting)**
None present or none present in regulated quantities.

**Clean Water Act Section 311 Hazardous Substances (40 CFR 117.3)**

| Chemical Identity | Reportable quantity |
|---|---|
| Hypochlorous acid, sodium salt (1:1) | Reportable quantity: 100 lbs. |
| Sodium hydroxide (Na(OH)) | Reportable quantity: 1000 lbs. |

**Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130):**
None present or none present in regulated quantities.

**US State Regulations**

**US. California Proposition 65**
No ingredient requiring a warning under CA Prop 65.

**US. New Jersey Worker and Community Right-to-Know Act**

| Chemical Identity |
|---|
| Hypochlorous acid, sodium salt (1:1) |

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**US. Massachusetts RTK - Substance List**
No ingredient regulated by MA Right-to-Know Law present.

**US. Pennsylvania RTK - Hazardous Substances**
No ingredient regulated by PA Right-to-Know Law present.

**US. Rhode Island RTK**
No ingredient regulated by RI Right-to-Know Law present.

## 16.Other information, including date of preparation or last revision

| | |
|---|---|
| **Issue Date:** | 05/06/2020 |
| **Version #:** | 3.2 |
| **Revision Information:** | |
| **Source of information:** | European Chemicals Agency (ECHA): Information on Chemicals. |
| **Further Information:** | No data available. |
| **Disclaimer:** | Disclaimer:<br>The information contained herein has been obtained from various sources and is believed to be correct as of the date issued. However, neither BD nor any of its subsidiaries assumes any liabilities whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability for a particular use of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist. BD provides SDS in electronic form so the information may be more easily accessed. Due to the possibility of errors during transmission, BD makes no representations as to the completeness or accuracy of the information. |

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# SAFETY DATA SHEET

## 1. Identification

**Product identifier**

| Product No.: | Product name: | Common name(s), synonym(s) |
|---|---|---|
| 660585 | BD™ Detergent Solution Concentrate | |

**Other means of identification**

**SDS number:** 088100200356

**Recommended use and restriction on use**

**Recommended use:** Scientific and Industrial laboratory use.
**Restrictions on use:** None known.

**Manufacturer/Importer/Supplier/Distributor Information**

**Manufacturer**

| | |
|---|---|
| Company Name: | Becton, Dickinson and Company - BD Biosciences |
| Address: | 2350 Qume Drive<br>95131 San Jose, CA USA |
| Telephone: | 1 877 232 8995 or 1 800 424 9300 |
| Fax: | |
| Contact Person: | Technical Services |
| E-mail: | ResearchApplications@bd.com or ClinicalApplications@bd.com |

**Emergency telephone number:** ChemTrec 1 800 424 9300

## 2. Hazard(s) identification

**Hazard Classification**

**Health Hazards**

| | |
|---|---|
| Skin Corrosion/Irritation | Category 1A |
| Serious Eye Damage/Eye Irritation | Category 1 |

**Label Elements**

**Hazard Symbol:**

**Signal Word:** Danger

**Hazard Statement:** H314: Causes severe skin burns and eye damage.

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**Precautionary Statements**

**Prevention:**
P260: Do not breathe dust/fume/gas/mist/vapors/spray.
P264: Wash thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.

**Response:**
P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P310: Immediately call a POISON CENTER/doctor.
P321: Specific treatment (see on this label).
P363: Wash contaminated clothing before reuse.

**Storage:**
P405: Store locked up.

**Disposal:**
P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

**Other hazards which do not result in GHS classification:**
None.

## 3. Composition/information on ingredients

**Mixtures**

| Chemical Identity | Common name and synonyms | CAS number | Content in percent (%)* |
|---|---|---|---|
| Acetic acid, 2-hydroxy- | | 79-14-1 | 10% |

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

## 4. First-aid measures

**General information:**
Causes severe skin burns and eye damage. Get immediate medical advice/attention.

**Ingestion:**
Call a physician or poison control center immediately. Rinse mouth thoroughly. Do not induce vomiting. If vomiting occurs, the head should be kept low so that stomach vomit doesn't enter the lungs.

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| | |
|---|---|
| **Inhalation:** | Move to fresh air. Get medical attention if any discomfort continues. |
| **Skin Contact:** | Take off immediately all contaminated clothing. Rinse skin with water [or shower]. Get medical attention promptly if symptoms occur after washing. |
| **Eye contact:** | Important! Immediately rinse with water for 60 minutes. Get medical attention immediately. Continue to rinse. |

**Most important symptoms/effects, acute and delayed**

| | |
|---|---|
| **Symptoms:** | Symptoms may be delayed. |
| **Hazards:** | Causes severe skin burns and eye damage. |

**Indication of immediate medical attention and special treatment needed**

| | |
|---|---|
| **Treatment:** | IF exposed or concerned: Get medical advice/attention. |

## 5. Fire-fighting measures

| | |
|---|---|
| **General Fire Hazards:** | Extinguish all ignition sources. Avoid sparks, flames, heat and smoking. Ventilate. Use water to keep fire exposed containers cool and disperse vapors. |

**Suitable (and unsuitable) extinguishing media**

| | |
|---|---|
| **Suitable extinguishing media:** | Use water fog, alcohol-resistant foam, dry chemical or carbon dioxide (CO2) to extinguish flames. |
| **Unsuitable extinguishing media:** | Do not use water jet as an extinguisher, as this will spread the fire. |
| **Specific hazards arising from the chemical:** | Fire or excessive heat may produce hazardous decomposition products. |

**Special protective equipment and precautions for firefighters**

| | |
|---|---|
| **Special fire fighting procedures:** | No unusual fire or explosion hazards noted. |
| **Special protective equipment for fire-fighters:** | Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA. |

## 6. Accidental release measures

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| | |
|---|---|
| **Personal precautions, protective equipment and emergency procedures:** | Ensure suitable personal protection (including respiratory protection) during removal of spillages in a confined area. Ventilate closed spaces before entering them. Avoid breathing mists or vapors. Keep unauthorized personnel away. |
| **Methods and material for containment and cleaning up:** | Stop leak if possible without any risk. Prevent runoff from entering drains, sewers, or streams. Dike far ahead of larger spills for later disposal. Absorb in vermiculite, dry sand or earth and place into containers. See Section 8 of the SDS for Personal Protective Equipment. For waste disposal, see section 13 of the SDS. |
| **Environmental Precautions:** | Do not contaminate water sources or sewer. |

## 7. Handling and storage

| | |
|---|---|
| **Precautions for safe handling:** | Avoid contact with eyes and prolonged or repeated contact with skin. Avoid inhalation of vapors and spray mists. Observe good industrial hygiene practices. Wear appropriate personal protective equipment. Provide good ventilation. |
| **Conditions for safe storage, including any incompatibilities:** | Store in original tightly closed container. Store in a cool, dry place with adequate ventilation. Keep away from incompatible materials, open flames, and high temperatures. |

## 8. Exposure controls/personal protection

**Control Parameters**

**Occupational Exposure Limits**

None of the components have assigned exposure limits.

| | |
|---|---|
| **Appropriate Engineering Controls** | Adequate ventilation should be provided so that exposure limits are not exceeded. Eye wash facilities and emergency shower must be available when handling this product. |

**Individual protection measures, such as personal protective equipment**

| | |
|---|---|
| **General information:** | Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing to remove contaminants. Discard contaminated footwear that cannot be cleaned. |
| **Eye/face protection:** | Wear safety glasses with side shields (or goggles) and a face shield. |
| **Skin Protection** | |
| **Hand Protection:** | Suitable gloves can be recommended by the glove supplier. |
| **Other:** | Chemical resistant clothing |
| **Respiratory Protection:** | In case of inadequate ventilation use suitable respirator. |

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**Hygiene measures:** Observe good industrial hygiene practices. Wash at the end of each work shift and before eating, smoking and using the toilet.

## 9. Physical and chemical properties

**Appearance**

| | |
|---|---|
| **Physical state:** | liquid |
| **Form:** | No data available. |
| **Color:** | Pale yellow |
| **Odor:** | Odorless |
| **Odor threshold:** | No data available. |
| **pH:** | 2.5 |
| **Melting point/freezing point:** | No data available. |
| **Initial boiling point and boiling range:** | No data available. |
| **Flash Point:** | No data available. |
| **Evaporation rate:** | No data available. |
| **Flammability (solid, gas):** | No data available. |
| **Upper/lower limit on flammability or explosive limits** | |
| **Flammability limit - upper (%):** | No data available. |
| **Flammability limit - lower (%):** | No data available. |
| **Explosive limit - upper (%):** | No data available. |
| **Explosive limit - lower (%):** | No data available. |
| **Vapor pressure:** | No data available. |
| **Vapor density:** | No data available. |
| **Relative density:** | No data available. |
| **Solubility(ies)** | |
| **Solubility in water:** | Very Soluble |
| **Solubility (other):** | No data available. |
| **Partition coefficient (n-octanol/water):** | No data available. |
| **Auto-ignition temperature:** | No data available. |
| **Decomposition temperature:** | No data available. |
| **Viscosity:** | No data available. |

## 10. Stability and reactivity

**Reactivity:** Product is not reactive under normal conditions and recommended use.

**Chemical Stability:** No data available.

**Possibility of hazardous reactions:** Stable; however, may decompose if heated.

**Conditions to avoid:** Avoid exposure to high temperatures or direct sunlight. Do not freeze.

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| | |
|---|---|
| **Incompatible Materials:** | Avoid contact with oxidizers or reducing agents. |
| **Hazardous Decomposition Products:** | By heating and fire, corrosive vapors/gases may be formed. |

## 11. Toxicological information

**Information on likely routes of exposure**

| | |
|---|---|
| **Ingestion:** | No data available. |
| **Inhalation:** | No data available. |
| **Skin Contact:** | No data available. |
| **Eye contact:** | No data available. |

**Symptoms related to the physical, chemical and toxicological characteristics**

| | |
|---|---|
| **Ingestion:** | No data available. |
| **Inhalation:** | No data available. |
| **Skin Contact:** | No data available. |
| **Eye contact:** | No data available. |

**Information on toxicological effects**

**Acute toxicity (list all possible routes of exposure)**

**Oral**
**Product:** No data available.

**Dermal**
**Product:** No data available.

**Inhalation**
**Product:** No data available.

**Repeated dose toxicity**
**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Acetic acid, 2-hydroxy- | LOAEL (Rat(Female, Male), Oral, 90 - 131 d): 300 mg/kg Oral Experimental result, Key study<br>NOAEL (Rat(Male), Oral, 90 - 131 d): 150 mg/kg Oral Experimental result, Key study<br>NOAEL (Rat(Male), Inhalation): 0.23 mg/l Inhalation Experimental result, Supporting study<br>NOAEL (Rat(Female), Oral, 90 - 131 d): 600 mg/kg Oral Experimental result, |

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Key study
NOAEL (Rat(Female, Male), Oral, 90 - 131 d): 600 mg/kg Oral Experimental result, Key study

**Skin Corrosion/Irritation**

**Product:** No data available.

**Specified substance(s):**

Acetic acid, 2-hydroxy- in vivo (Rabbit): Corrosive Experimental result, Key study

**Serious Eye Damage/Eye Irritation**

**Product:** No data available.

**Respiratory or Skin Sensitization**

**Product:** No data available.

**Specified substance(s):**

Acetic acid, 2-hydroxy- Skin sensitization:, in vivo (Guinea pig): Non sensitising

**Carcinogenicity**

**Product:** No data available.

**IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:**

No carcinogenic components identified

**US. National Toxicology Program (NTP) Report on Carcinogens:**

No carcinogenic components identified

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050):**

No carcinogenic components identified

**Germ Cell Mutagenicity**

**In vitro**

**Product:** No data available.

**In vivo**

**Product:** No data available.

**Reproductive toxicity**

**Product:** No data available.

**Specific Target Organ Toxicity - Single Exposure**

**Product:** No data available.

**Specific Target Organ Toxicity - Repeated Exposure**

**Product:** No data available.

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**Aspiration Hazard**
**Product:** No data available.

**Other effects:** No data available.

## 12. Ecological information

**Ecotoxicity:**

**Acute hazards to the aquatic environment:**

**Fish**
**Product:** Not expected to be harmful to aquatic organisms.

**Aquatic Invertebrates**
**Product:** No data available.

**Specified substance(s):**
Acetic acid, 2-hydroxy- EC 50 (Daphnia magna, 48 h): 141 mg/l Experimental result, Key study
EC 50 (Daphnia magna, 24 h): 141 mg/l Experimental result, Key study
NOAEL (Daphnia magna, 48 h): 100 mg/l Experimental result, Key study

**Chronic hazards to the aquatic environment:**

**Fish**
**Product:** No data available.

**Aquatic Invertebrates**
**Product:** No data available.

**Toxicity to Aquatic Plants**
**Product:** No data available.

**Persistence and Degradability**

**Biodegradation**
**Product:** No data available.

**Specified substance(s):**
Acetic acid, 2-hydroxy- 78 % (11 d) Detected in water. Experimental result, Key study
50 % (2 d) Sediment Experimental result, Supporting study
89.6 % (7 d) Detected in water. Experimental result, Supporting study
96 % (28 d) Sediment Experimental result, Supporting study
10 % (1 d) Sediment Experimental result, Supporting study

**BOD/COD Ratio**

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**Product:** No data available.

**Bioaccumulative potential**

**Bioconcentration Factor (BCF)**

**Product:** No data available.

**Partition Coefficient n-octanol / water (log Kow)**

**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Acetic acid, 2-hydroxy- | Log Kow: -1.11 |

**Mobility in soil:** No data available.

**Known or predicted distribution to environmental compartments**

| | |
|---|---|
| Acetic acid, 2-hydroxy- | No data available. |

**Other adverse effects:** No data available.

## 13. Disposal considerations

**General information:** Dispose of waste and residues in accordance with local authority requirements.

**Disposal instructions:** This material and/or its container must be disposed of as hazardous waste.

**Contaminated Packaging:** No data available.

## 14. Transport information

| | |
|---|---|
| **DOT**UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Label(s): | Not regulated. |
| Packing Group: | Not regulated. |
| Marine Pollutant: | Not regulated. |
| Limited quantity | Not regulated. |
| Excepted quantity | Not regulated. |

Special precautions for user: Not regulated.

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**IMDG**

| | |
|---|---|
| UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| EmS No.: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine Pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

**IATA**

| | |
|---|---|
| UN Number: | Not regulated. |
| Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es): | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

## 15. Regulatory information

**US Federal Regulations**

**TSCA Section 12(b) Export Notification (40 CFR 707, Subpt. D)**
None present or none present in regulated quantities.

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)**
None present or none present in regulated quantities.

**CERCLA Hazardous Substance List (40 CFR 302.4):**
None present or none present in regulated quantities.

**Superfund Amendments and Reauthorization Act of 1986 (SARA)**

**Hazard categories**
Immediate (Acute) Health Hazards
Skin Corrosion or Irritation
Serious eye damage or eye irritation

**SARA 302 Extremely Hazardous Substance**
None present or none present in regulated quantities.

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**SARA 304 Emergency Release Notification**
None present or none present in regulated quantities.

**SARA 311/312 Hazardous Chemical**

| Chemical Identity | Threshold Planning Quantity |
|---|---|
| Acetic acid, 2-hydroxy- | 10000 lbs |

**SARA 313 (TRI Reporting)**
None present or none present in regulated quantities.

**Clean Water Act Section 311 Hazardous Substances (40 CFR 117.3)**
None present or none present in regulated quantities.

**Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130):**
None present or none present in regulated quantities.

**US State Regulations**

**US. California Proposition 65**
No ingredient requiring a warning under CA Prop 65.

**US. New Jersey Worker and Community Right-to-Know Act**
No ingredient regulated by NJ Right-to-Know Law present.

**US. Massachusetts RTK - Substance List**
No ingredient regulated by MA Right-to-Know Law present.

**US. Pennsylvania RTK - Hazardous Substances**
No ingredient regulated by PA Right-to-Know Law present.

**US. Rhode Island RTK**
No ingredient regulated by RI Right-to-Know Law present.

## 16.Other information, including date of preparation or last revision

**Issue Date:** 03/29/2019

**Version #:** 1.1

**Revision Information:**

**Further Information:** No data available.

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**Disclaimer:**

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# SAFETY DATA SHEET

## 1. Identification

**Product identifier**

| Product No.: | Product name: | Common name(s), synonym(s) |
|---|---|---|
| 660586 | BD™ Extended Flow Cell Clean Solution | |

**Other means of identification**

**SDS number:** 088100200357

**Recommended use and restriction on use**

**Recommended use:** Reserved for industrial and professional use.
**Restrictions on use:** None known.

**Manufacturer/Importer/Supplier/Distributor Information**

**Manufacturer**

| | |
|---|---|
| Company Name: | Becton, Dickinson and Company - BD Biosciences |
| Address: | 2350 Qume Drive<br>95131 San Jose, CA USA |
| Telephone: | 1 877 232 8995 or 1 800 424 9300 |
| Fax: | |
| Contact Person: | Technical Services |
| E-mail: | ResearchApplications@bd.com or ClinicalApplications@bd.com |

**Emergency telephone number:** ChemTrec 1 800 424 9300

## 2. Hazard(s) identification

**Hazard Classification**

Not classified

**Label Elements**

**Hazard Symbol:** No symbol

**Signal Word:** No signal word.

**Hazard Statement:** Not applicable
**Precautionary Statements** Not applicable

**Other hazards which do not result in GHS classification:** None.

## 3. Composition/information on ingredients

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**Mixtures**

| Chemical Identity | Common name and synonyms | CAS number | Content in percent (%)* |
|---|---|---|---|
| Ethanol | | 64-17-5 | 4.7184% |
| Methanol | | 67-56-1 | 0.2483% |

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

## 4. First-aid measures

**General information:** Get medical attention if symptoms occur.

**Ingestion:** Call a physician or poison control center immediately. Only induce vomiting at the instruction of medical personnel. Never give anything by mouth to an unconscious person.

**Inhalation:** Provide fresh air, warmth and rest, preferably in comfortable upright sitting position.

**Skin Contact:** Wash contact areas with soap and water. Remove contaminated clothing. Launder contaminated clothing before reuse.

**Eye contact:** Immediately flush with plenty of water for at least 15 minutes. If easy to do, remove contact lenses.

**Most important symptoms/effects, acute and delayed**

**Symptoms:** No data available.

**Indication of immediate medical attention and special treatment needed**

**Treatment:** No data available.

## 5. Fire-fighting measures

**General Fire Hazards:** Extinguish all ignition sources. Avoid sparks, flames, heat and smoking. Ventilate. Use water spray to keep fire-exposed containers cool.

**Suitable (and unsuitable) extinguishing media**

**Suitable extinguishing media:** Use fire-extinguishing media appropriate for surrounding materials.

**Unsuitable extinguishing media:** Not applicable

**Specific hazards arising from the chemical:** Fire or excessive heat may produce hazardous decomposition products.

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**Special protective equipment and precautions for firefighters**

| | |
|---|---|
| **Special fire fighting procedures:** | No unusual fire or explosion hazards noted. |
| **Special protective equipment for fire-fighters:** | Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA. |

## 6. Accidental release measures

| | |
|---|---|
| **Personal precautions, protective equipment and emergency procedures:** | Contact local authorities in case of spillage to drain/aquatic environment. Ensure suitable personal protection (including respiratory protection) during removal of spillages in a confined area. |
| **Methods and material for containment and cleaning up:** | Absorb spillage with suitable absorbent material. Prevent runoff from entering drains, sewers, or streams. See Section 8 of the SDS for Personal Protective Equipment. For waste disposal, see section 13 of the SDS. |
| **Environmental Precautions:** | Avoid release to the environment. |

## 7. Handling and storage

| | |
|---|---|
| **Precautions for safe handling:** | When using do not eat, drink or smoke. Read and follow manufacturer's recommendations. Use personal protective equipment as required. |
| **Conditions for safe storage, including any incompatibilities:** | Store in a cool, dry place. Keep container tightly closed. |

## 8. Exposure controls/personal protection

**Control Parameters**

**Occupational Exposure Limits**

| Chemical Identity | Type | Exposure Limit Values | Source |
|---|---|---|---|
| Ethanol | TWA | 1,000 ppm 1,900 mg/m3 | US. OSHA Table Z-1-A (29 CFR 1910.1000) (1989) |
| | TWA | 1,000 ppm 1,900 mg/m3 | US. Tennessee. OELs. Occupational Exposure Limits, Table Z1A (06 2008) |
| | AN ESL | 1,000 ppb | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | ST ESL | 10,000 ppb | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | AN ESL | 1,880 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | ST ESL | 18,800 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | TWA PEL | 1,000 ppm 1,900 mg/m3 | US. California Code of Regulations, Title 8, Section 5155. Airborne Contaminants (08 |

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| | | | | |
|---|---|---|---|---|
| | | | | 2010) |
| | STEL | 1,000 ppm | | US. ACGIH Threshold Limit Values (12 2010) |
| | REL | 1,000 ppm | 1,900 mg/m3 | US. NIOSH: Pocket Guide to Chemical Hazards (2005) |
| | PEL | 1,000 ppm | 1,900 mg/m3 | US. OSHA Table Z-1 Limits for Air Contaminants (29 CFR 1910.1000) (02 2006) |
| Methanol | STEL | 250 ppm | 325 mg/m3 | US. OSHA Table Z-1-A (29 CFR 1910.1000) (1989) |
| | TWA | 200 ppm | 260 mg/m3 | US. OSHA Table Z-1-A (29 CFR 1910.1000) (1989) |
| | STEL | 250 ppm | 325 mg/m3 | US. Tennessee. OELs. Occupational Exposure Limits, Table Z1A (06 2008) |
| | TWA | 200 ppm | 260 mg/m3 | US. Tennessee. OELs. Occupational Exposure Limits, Table Z1A (06 2008) |
| | ST ESL | | 2,620 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | AN ESL | | 200 ppb | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | AN ESL | | 262 µg/m3 | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | ST ESL | | 2,000 ppb | US. Texas. Effects Screening Levels (Texas Commission on Environmental Quality) (12 2010) |
| | STEL | 250 ppm | 325 mg/m3 | US. California Code of Regulations, Title 8, Section 5155. Airborne Contaminants (08 2010) |
| | TWA PEL | 200 ppm | 260 mg/m3 | US. California Code of Regulations, Title 8, Section 5155. Airborne Contaminants (08 2010) |
| | Ceiling | 1,000 ppm | | US. California Code of Regulations, Title 8, Section 5155. Airborne Contaminants (08 2010) |
| | STEL | 250 ppm | | US. ACGIH Threshold Limit Values (12 2010) |
| | TWA | 200 ppm | | US. ACGIH Threshold Limit Values (12 2010) |
| | REL | 200 ppm | 260 mg/m3 | US. NIOSH: Pocket Guide to Chemical Hazards (2005) |
| | STEL | 250 ppm | 325 mg/m3 | US. NIOSH: Pocket Guide to Chemical Hazards (2005) |
| | PEL | 200 ppm | 260 mg/m3 | US. OSHA Table Z-1 Limits for Air Contaminants (29 CFR 1910.1000) (02 2006) |

**Biological Limit Values**

| Chemical Identity | Exposure Limit Values | Source |
|---|---|---|
| Methanol (methanol: Sampling time: End of shift.) | 15 mg/l (Urine) | ACGIH BEI (03 2013) |

**Appropriate Engineering Controls**

No special requirements under ordinary conditions of use and with adequate ventilation.

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**Individual protection measures, such as personal protective equipment**

| | |
|---|---|
| **General information:** | Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing to remove contaminants. Discard contaminated footwear that cannot be cleaned. |
| **Eye/face protection:** | Wear safety glasses with side shields (or goggles). |
| **Skin Protection** | |
| **Hand Protection:** | Chemical resistant gloves Suitable gloves can be recommended by the glove supplier. Wash hands after contact. |
| **Other:** | Wear a lab coat or similar protective clothing. |
| **Respiratory Protection:** | If engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn. |
| **Hygiene measures:** | Observe good industrial hygiene practices. |

## 9. Physical and chemical properties

**Appearance**

| | |
|---|---|
| **Physical state:** | liquid |
| **Form:** | Aqueous Solution |
| **Color:** | Colorless |
| **Odor:** | Characteristic |
| **Odor threshold:** | No data available. |
| **pH:** | No data available. |
| **Melting point/freezing point:** | No data available. |
| **Initial boiling point and boiling range:** | No data available. |
| **Flash Point:** | No data available. |
| **Evaporation rate:** | No data available. |
| **Flammability (solid, gas):** | No data available. |
| **Upper/lower limit on flammability or explosive limits** | |
| **Flammability limit - upper (%):** | No data available. |
| **Flammability limit - lower (%):** | No data available. |
| **Explosive limit - upper (%):** | No data available. |
| **Explosive limit - lower (%):** | No data available. |
| **Vapor pressure:** | No data available. |
| **Vapor density:** | No data available. |
| **Relative density:** | No data available. |
| **Solubility(ies)** | |
| **Solubility in water:** | No data available. |

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| | |
|---|---|
| **Solubility (other):** | No data available. |
| **Partition coefficient (n-octanol/water):** | No data available. |
| **Auto-ignition temperature:** | No data available. |
| **Decomposition temperature:** | No data available. |
| **Viscosity:** | No data available. |

## 10. Stability and reactivity

| | |
|---|---|
| **Reactivity:** | Stable under normal temperature conditions and recommended use. |
| **Chemical Stability:** | Material is stable under normal conditions. |
| **Possibility of hazardous reactions:** | Not determined. |
| **Conditions to avoid:** | Avoid exposure to high temperatures or direct sunlight. |
| **Incompatible Materials:** | Metals. Water reactive material. |
| **Hazardous Decomposition Products:** | Stable; however, may decompose if heated. |

## 11. Toxicological information

**General information:** No data on possible toxicity effects have been found.

**Information on likely routes of exposure**

| | |
|---|---|
| **Ingestion:** | No harmful effects expected in amounts likely to be ingested by accident. |
| **Inhalation:** | Limited inhalation hazard at normal work temperatures. |
| **Skin Contact:** | Negligible irritation to skin at ambient temperatures. |
| **Eye contact:** | Elevated temperatures or mechanical action may form vapors, mist, or fumes which may be irritating to the eyes, nose, throat, or lungs. |

**Symptoms related to the physical, chemical and toxicological characteristics**

| | |
|---|---|
| **Ingestion:** | No data available. |
| **Inhalation:** | No data available. |
| **Skin Contact:** | No data available. |
| **Eye contact:** | No data available. |

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## Information on toxicological effects

### Acute toxicity (list all possible routes of exposure)

**Oral**
**Product:** ATEmix: 40,273.86 mg/kg

**Dermal**
**Product:** ATEmix: 120,821.59 mg/kg

**Inhalation**
**Product:** ATEmix: 1,208.22 mg/l

**Repeated dose toxicity**
**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Ethanol | Based on available data, the classification criteria are not met.<br>LOAEL (Rat(Female, Male), Inhalation, 7,318 - 7,496 h): 1.3 mg/l Inhalation Read-across from supporting substance (structural analogue or surrogate), Weight of Evidence study<br>NOAEL (Guinea pig, Inhalation, 10.5 Weeks): 3,000 ppm(m) Inhalation Experimental result, Supporting study<br>LOAEL (Rat(Male), Inhalation, 1 - 6 Weeks): 13.3 mg/l Inhalation Read-across from supporting substance (structural analogue or surrogate), Supporting study<br>LOAEL (Monkey, Inhalation, 5 - 20 d): 3.99 mg/l Inhalation Read-across from supporting substance (structural analogue or surrogate), Supporting study |
| Methanol | NOAEL (Rat(Female, Male), Inhalation): 6.66 mg/l Inhalation Experimental result, Weight of Evidence study<br>LOAEL (Rat(Male), Inhalation, 1 - 6 Weeks): 13.3 mg/l Inhalation Experimental result, Supporting study<br>NOAEL (Rat(Male), Inhalation, 1 - 6 Weeks): 2.65 mg/l Inhalation Experimental result, Supporting study<br>NOAEL (Rat(Male), Inhalation, 1 - 6 Weeks): 0.26 mg/l Inhalation Experimental result, Supporting study<br>NOAEL (Rat(Female, Male), Inhalation, 7,318 - 7,496 h): 0.13 mg/l Inhalation Experimental result, Weight of Evidence study |

**Skin Corrosion/Irritation**
**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Ethanol | in vivo (Rabbit): Not irritant Experimental result, Key study |

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| | |
|---|---|
| Methanol | in vivo (Rabbit): Not irritant Experimental result, Key study |

**Serious Eye Damage/Eye Irritation**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | in vivo (Rabbit, 24 - 72 hrs): Not irritating EU |
| Methanol | in vivo (Rabbit, 24 - 72 hrs): Not irritating |

**Respiratory or Skin Sensitization**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met.<br>Skin sensitization:, in vivo (Guinea pig): Non sensitising |
| Methanol | Skin sensitization:, in vivo (Guinea pig): Non sensitising |

**Carcinogenicity**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met. |

**IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:**
No carcinogenic components identified

**US. National Toxicology Program (NTP) Report on Carcinogens:**
No carcinogenic components identified

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050):**
No carcinogenic components identified

**Germ Cell Mutagenicity**

**In vitro**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met. |

**In vivo**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met. |

**Reproductive toxicity**

| | |
|---|---|
| **Product:** | No data available. |

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**Specified substance(s):**

| | |
|---|---|
| Ethanol | Based on available data, the classification criteria are not met. |

**Specific Target Organ Toxicity - Single Exposure**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met. |
| Methanol | Oral: Nervous System - Causes damage to organs. |

**Specific Target Organ Toxicity - Repeated Exposure**

| | |
|---|---|
| **Product:** | No data available. |
| **Specified substance(s):** | |
| Ethanol | Based on available data, the classification criteria are not met. |

**Aspiration Hazard**

| | |
|---|---|
| **Product:** | No data available. |

**Other effects:** No data available.

## 12. Ecological information

**Ecotoxicity:**

**Acute hazards to the aquatic environment:**

**Fish**
**Product:** No negative effects on the aquatic environment are known.

**Aquatic Invertebrates**
**Product:** No negative effects on the aquatic environment are known.

**Chronic hazards to the aquatic environment:**

**Fish**
**Product:** No negative effects on the aquatic environment are known.

**Aquatic Invertebrates**
**Product:** No negative effects on the aquatic environment are known.

**Toxicity to Aquatic Plants**
**Product:** No negative effects on the aquatic environment are known.

**Persistence and Degradability**

**Biodegradation**
**Product:** Expected to be readily biodegradable.

**BOD/COD Ratio**
**Product:** No data available.

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**Bioaccumulative potential**

**Bioconcentration Factor (BCF)**

**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Ethanol | Potential to bioaccumulate is low.<br>Cyprinus carpio, Bioconcentration Factor (BCF): 4.5 Aquatic sediment Read-across from supporting substance (structural analogue or surrogate), Supporting study<br>Cyprinus carpio, Bioconcentration Factor (BCF): 3 Aquatic sediment Read-across from supporting substance (structural analogue or surrogate), Supporting study<br>Leuciscus idus, Bioconcentration Factor (BCF): 0.2 Aquatic sediment Read-across from supporting substance (structural analogue or surrogate), Not specified<br>Cyprinus carpio, Bioconcentration Factor (BCF): 1 Aquatic sediment Read-across from supporting substance (structural analogue or surrogate), Supporting study |
| Methanol | Leuciscus idus, Bioconcentration Factor (BCF): < 10 Aquatic sediment Experimental result, Supporting study<br>Cyprinus carpio, Bioconcentration Factor (BCF): 4.5 Aquatic sediment Experimental result, Supporting study<br>Cyprinus carpio, Bioconcentration Factor (BCF): 1 Aquatic sediment Experimental result, Supporting study<br>Cyprinus carpio, Bioconcentration Factor (BCF): 3 Aquatic sediment Experimental result, Supporting study<br>Green algae (Chlorella fusca vacuolata), Bioconcentration Factor (BCF): 28,400 (Static) |

**Partition Coefficient n-octanol / water (log Kow)**

**Product:** No data available.

**Specified substance(s):**

| | |
|---|---|
| Ethanol | Log Kow: -0.31 |
| Methanol | Log Kow: -0.77 |

**Mobility in soil:** No data available.

**Known or predicted distribution to environmental compartments**

| | |
|---|---|
| Ethanol | soil - Very mobile liquid |
| Methanol | No data available. |

**Other adverse effects:** The product is not expected to be hazardous to the environment.

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## 13. Disposal considerations

| | |
|---|---|
| **General information:** | Dispose of waste and residues in accordance with local authority requirements. |
| **Disposal instructions:** | Dispose of waste at an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal. |
| **Contaminated Packaging:** | No data available. |

## 14. Transport information

| | |
|---|---|
| **DOT**UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Label(s): | Not regulated. |
| Packing Group: | Not regulated. |
| Marine Pollutant: | Not regulated. |
| Limited quantity | Not regulated. |
| Excepted quantity | Not regulated. |
| Special precautions for user: | Not regulated. |

**IMDG**

| | |
|---|---|
| UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| EmS No.: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine Pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

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**IATA**

| | |
|---|---|
| UN Number: | Not regulated. |
| Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es): | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

## 15. Regulatory information

**US Federal Regulations**

**TSCA Section 12(b) Export Notification (40 CFR 707, Subpt. D)**
None present or none present in regulated quantities.

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)**
None present or none present in regulated quantities.

**CERCLA Hazardous Substance List (40 CFR 302.4):**

| Chemical Identity | Reportable quantity |
|---|---|
| Ethanol | 100 lbs. |
| Methanol | 5000 lbs. |

**Superfund Amendments and Reauthorization Act of 1986 (SARA)**

**Hazard categories**
Not classified
Not classified

**SARA 302 Extremely Hazardous Substance**
None present or none present in regulated quantities.

**SARA 304 Emergency Release Notification**

| Chemical Identity | Reportable quantity |
|---|---|
| Ethanol | 100 lbs. |
| Methanol | 5000 lbs. |

**SARA 311/312 Hazardous Chemical**

| Chemical Identity | Threshold Planning Quantity |
|---|---|
| Ethanol | 10000 lbs |
| Methanol | 10000 lbs |

**SARA 313 (TRI Reporting)**
None present or none present in regulated quantities.

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**Clean Water Act Section 311 Hazardous Substances (40 CFR 117.3)**
None present or none present in regulated quantities.

**Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130):**
None present or none present in regulated quantities.

**US State Regulations**

**US. California Proposition 65**

**WARNING:** This product can expose you to chemicals including, Ethanol, which is [are] known to the State of California to cause cancer and birth defects or other reproductive harm.
This product can expose you to chemicals including, Methanol, which is [are] known to the State of California to cause birth defects or other reproductive harm.
For more information go to www.P65Warnings.ca.gov.

**US. New Jersey Worker and Community Right-to-Know Act**

**Chemical Identity**
Ethanol

**US. Massachusetts RTK - Substance List**

**Chemical Identity**
Ethanol

**US. Pennsylvania RTK - Hazardous Substances**

**Chemical Identity**
Ethanol

**US. Rhode Island RTK**

**Chemical Identity**
Ethanol

## 16.Other information, including date of preparation or last revision

| | |
|---|---|
| **Issue Date:** | 03/29/2019 |
| **Version #:** | 1.1 |
| **Revision Information:** | |
| **Source of information:** | European Chemicals Agency (ECHA): Information on Chemicals. |
| **Further Information:** | No data available. |

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# BD FACS™ Lysing Solution

Catalog No. 349202

23-1358(14)
2023-04
English

℞ Only CE IVD

## 1. INTENDED USE

BD FACS™ Lysing Solution is intended for lysing red blood cells for flow cytometric applications. It can be used in both lyse/wash and lyse/no-wash procedures.

## 2. SUMMARY OF THE TEST

Efficient detection of leukocytes in specimens depends on the elimination of interfering cells. Whole blood lysis has been shown to be as effective as density gradient centrifugation in the preparation of peripheral blood mononuclear cells (PBMCs) for lymphocyte subset analysis.[1,2,3,4] In clinical laboratories, whole blood lysis methods have essentially replaced Ficoll-Paque™ density gradient separation because of shorter sample preparation time and less handling of whole blood.[5] Studies have also shown that the lysed whole blood method is less likely to show loss of leukocyte subsets and may help improve assay reproducibility when compared to earlier methods.[5,6,7]

BD FACS™ Lysing Solution is intended for use by laboratory professionals.

### Principle of Operation

When the specimen is added to the antibody reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. The stained samples are then treated with BD FACS™ Lysing Solution, which lyses red blood cells (RBCs) under gentle hypotonic conditions while preserving the leukocytes.

## 3. REAGENT

### Reagent Composition

BD FACS™ Lysing Solution is a proprietary buffered solution containing formaldehyde and diethylene glycol.

### Precautions

BD FACS™ Lysing Solution contains 31.34% ethanol, 2,2´-oxybis- (diethylene glycol) (CAS number 111-46-6, EC number 203-872-2), 9.77% formaldehyde (CAS number 50-00-0, EC number 200-001-8), and 3.43% methanol (CAS number 67-56-1, EC number 200-659-6). The lysing solution is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Regulation (EC) No 1272/2008, and 29 CFR 1910.1200. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

| | **Danger** |
|---|---|
|  | H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled.<br>H314: Causes severe skin burns and eye damage.<br>H317: May cause an allergic skin reaction.<br>H335: May cause respiratory irritation.<br>H341: Suspected of causing genetic defects.<br>H350: May cause cancer.<br>H370: Causes damage to organs.<br>H373: May cause damage to organs through prolonged or repeated exposure.<br>US only: H402: Harmful to aquatic life. |
| **Prevention** | P201: Obtain special instructions before use.<br>P202: Do not handle until all safety precautions have been read and understood.<br>P260: Do not breathe dust/fume/gas/mist/vapors/spray.<br>P264: Wash face, hands and any exposed skin thoroughly after handling.<br>P270: Do not eat, drink or smoke when using this product.<br>P271: Use only outdoors or in a well-ventilated area.<br>P272: Contaminated work clothing should not be allowed out of the workplace.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves/protective clothing/eye protection/face protection. |
| **Response** | P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.<br>P312: Call a POISON CENTER or doctor/physician if you feel unwell.<br>P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].<br>P363: Wash contaminated clothing before reuse.<br>P333+P313: If skin irritation or rash occurs: Get medical advice/attention.<br>P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing.<br>P310: Immediately call a POISON CENTER/doctor.<br>P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.<br>P307+P311: IF exposed: Call a POISON CENTER or doctor/ physician.<br>P308+P313: If exposed or concerned: Get medical advice/attention. |
| **Storage** | P405: Store locked up. |
| **Disposal** | P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations. |

### Storage and Handling

- BD FACS™ Lysing Solution (10X) is stable until the expiration date shown on the bottle label when stored as directed.
- The storage temperature is 2–25 °C.
- Do not use this reagent if discoloration occurs or a precipitate forms.

## 4. INSTRUMENT

BD FACS™ Lysing Solution is designed for flow cytometers equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect forward scatter (FSC) and side scatter (SSC).

## 5. SPECIMEN COLLECTION AND PREPARATION

See the instructions for use (IFU) for the reagent you are using for information about specimens supported.

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[8,9] 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.

## 6. PROCEDURE

### Reagents and Materials

### Reagents and materials provided

BD FACS™ Lysing Solution is provided as 100 mL of a 10X concentrate. After dilution, this volume is sufficient for 2,000 tests when used in lyse/no-wash procedures or for 500 tests when used in lyse/wash procedures.

### Reagents and materials required but not provided

- 1X BD FACS™ Lysing Solution, diluted as described
- BD fluorochrome-conjugated antibodies to human leukocyte antigens
- Vortex mixer
- Micropipettor with tips
- Other materials might be required. Refer to the appropriate reagent IFU for more information.

### 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.

### Staining the Specimen

Stain the specimen following instructions in the appropriate reagent IFU. Lyse RBCs as directed using diluted (1X) BD FACS™ Lysing Solution.

## 7. LIMITATIONS

- Samples with nucleated erythrocytes show incomplete lysis of RBCs because BD FACS™ Lysing Solution does not lyse nucleated erythrocytes as efficiently as enucleated RBCs. This may also occur when assaying blood samples from patients with certain hematologic disorders in which RBCs are difficult to lyse, as in myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.[7,8]
- BD FACS™ Lysing Solution was developed for use with BD flow cytometers.
- BD FACS™ Lysing Solution was developed using EDTA as the anticoagulant. BD has limited information concerning use of other anticoagulants such as heparin.

## REFERENCES


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3. Renzi P, Ginns LC. Analysis of T cell subsets in normal adults: comparison of whole blood lysis technique to Ficoll-Hypaque separation by flow cytometry. *J Immunol Methods*. 1987;98:53-56.
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7. Landay AL, Muirhead KA. Procedural guidelines for performing immunophenotyping by flow cytometry. *Clin Immunol Immunopath*. 1989;52:48-60.
8. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
9. 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.


## NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

## 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.

## HISTORY

| Revision | Date | Changes made |
|---|---|---|
| 23-1358(13) | 2021-11 | Updated to meet requirements of Regulation (EU) 2017/746. |
| 23-1358(14) | 2023-04 | Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary. Added Rx only symbol. |

**Symbols Glossary**

Please refer to product labeling for applicable symbols.

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|  | Authorized representative in the European Community |
|  | Authorised representative in Switzerland |
|  | Date of manufacture |
|  | Use-by date |
|  | Batch code |
|  | Catalogue number |
|  | Serial number |
|  | Sterile |
|  | Sterilized using aseptic processing techniques |
|  | Sterilized using ethylene oxide |
|  | Sterilized using irradiation |
|  | Sterilized using steam or dry heat |
|  | Do not resterilize |
|  | Non-sterile |
|  | Do not use if package is damaged and consult *instructions for use* |
|  | Sterile fluid path |
|  | Sterile fluid path (ethylene oxide) |
|  | 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 |
|  | Contains or presence of natural rubber latex |
|  | In vitro diagnostic medical device |
|  | Negative 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 |
|  | Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP) |
|  | Collect separately<br>Indicates separate collection for waste of electrical and electronic equipment required. |
|  | CE marking; Signifies European technical conformity |
|  | Device for near-patient testing |
|  | Device for self-testing |
|  | This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner." |
|  | Country of manufacture<br>"CC" shall be replaced by either the two letter or the three letter country code. |
|  | Collection time |
|  | Cut |
|  | Peel here |
|  | Collection date |
|  | Keep away from light |
|  | Hydrogen gas is generated |
|  | Perforation |
|  | Start panel sequence number |
|  | End panel sequence number |
|  | Internal sequence number |
|  | <Box #> / <Total Boxes> |
|  | Medical device |
|  | Contains hazardous substances |
|  | Ukrainian conformity mark |
|  | Meets FCC requirements per 21 CFR Part 15 |
|  | UL product certification for US and Canada |
|  | Unique device identifier |
|  | Importer |
|  | Place patient label in framed area only |
|  | Magnetic resonance (MR) safe |
|  | Magnetic resonance (MR) conditional |
|  | Magnetic resonance (MR) unsafe |
|  | For use with |
|  | This Product Contains Dry Natural Rubber |
|  | For Export Only |
|  | Instruments |

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

L006715(08) 2023-03

## CONTACT INFORMATION


**Becton, Dickinson and Company**
**BD Biosciences**
155 North McCarthy Boulevard
Milpitas, California 95035 USA

EC REP **Becton Dickinson Ireland Ltd.**
Donore Road, Drogheda
Co. Louth, A92 YW26
Ireland

**Becton Dickinson Distribution Center NV**
Laagstraat 57
9140 Temse, Belgium

CH REP **BD Switzerland Sàrl**
Route de Crassier 17
Business Park Terre-Bonne
Bâtiment A4
1262 Eysins
Switzerland

**Becton Dickinson AG**
Binningerstrasse 94
4123 Allschwil
Switzerland

**BD Biosciences**
**European Customer Support**
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand Distributors:

**Becton Dickinson Pty Ltd.**
66 Waterloo Road
Macquarie Park NSW 2113
Australia

**Becton Dickinson Limited**
14B George Bourke Drive
Mt. Wellington Auckland 1060
New Zealand


Technical Service and Support: In the United States contact BD at 1.877.232.8995 or bdbiosciences.com.
For regions outside the United States, contact your local BD representative or bdbiosciences.com.

ClinicalApplications@bd.com

eifu.bd.com

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07417 USA
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# SAFETY DATA SHEET

## 1. Identification

**Product identifier**

| Product No.: | Product name: | Common name(s), synonym(s) |
|---|---|---|
| 660584 | BD™ Sheath Additive | |

**Other means of identification**
**SDS number:** 088100200355

**Recommended use and restriction on use**

**Recommended use:** Reserved for industrial and professional use.
**Restrictions on use:** None known.

**Manufacturer/Importer/Supplier/Distributor Information**

**Manufacturer**

| | |
|---|---|
| Company Name: | Becton, Dickinson and Company - BD Biosciences |
| Address: | 2350 Qume Drive<br>95131 San Jose, CA USA |
| Telephone: | 1 877 232 8995 or 1 800 424 9300 |
| Fax: | |
| Contact Person: | Technical Services |
| E-mail: | ResearchApplications@bd.com or ClinicalApplications@bd.com |

**Emergency telephone number:** ChemTrec 1 800 424 9300

## 2. Hazard(s) identification

**Hazard Classification**

Not classified

**Label Elements**

| | |
|---|---|
| **Hazard Symbol:** | No symbol |
| **Signal Word:** | No signal word. |
| **Hazard Statement:** | Not applicable |
| **Precautionary Statements** | Not applicable |

**Other hazards which do not result in GHS classification:** None.

## 3. Composition/information on ingredients

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**Mixtures**

| Chemical Identity | Common name and synonyms | CAS number | Content in percent (%)* |
|---|---|---|---|
| Sodium fluoride (NaF) | | 7681-49-4 | 0.82% |

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

## 4. First-aid measures

**General information:** Get medical attention if symptoms occur.

**Ingestion:** Call a physician or poison control center immediately. Only induce vomiting at the instruction of medical personnel. Never give anything by mouth to an unconscious person.

**Inhalation:** Provide fresh air, warmth and rest, preferably in comfortable upright sitting position.

**Skin Contact:** Wash contact areas with soap and water. Remove contaminated clothing. Launder contaminated clothing before reuse.

**Eye contact:** Immediately flush with plenty of water for at least 15 minutes. If easy to do, remove contact lenses.

**Most important symptoms/effects, acute and delayed**

**Symptoms:** No data available.

**Indication of immediate medical attention and special treatment needed**

**Treatment:** No data available.

## 5. Fire-fighting measures

**General Fire Hazards:** Extinguish all ignition sources. Avoid sparks, flames, heat and smoking. Ventilate. Use water spray to keep fire-exposed containers cool.

**Suitable (and unsuitable) extinguishing media**

**Suitable extinguishing media:** Use fire-extinguishing media appropriate for surrounding materials.

**Unsuitable extinguishing media:** Not applicable

**Specific hazards arising from the chemical:** Fire or excessive heat may produce hazardous decomposition products.

**Special protective equipment and precautions for firefighters**

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| | |
|---|---|
| **Special fire fighting procedures:** | No unusual fire or explosion hazards noted. |
| **Special protective equipment for fire-fighters:** | Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA. |

## 6. Accidental release measures

| | |
|---|---|
| **Personal precautions, protective equipment and emergency procedures:** | Contact local authorities in case of spillage to drain/aquatic environment. Ensure suitable personal protection (including respiratory protection) during removal of spillages in a confined area. |
| **Methods and material for containment and cleaning up:** | Absorb spillage with suitable absorbent material. Prevent runoff from entering drains, sewers, or streams. See Section 8 of the SDS for Personal Protective Equipment. For waste disposal, see section 13 of the SDS. |
| **Environmental Precautions:** | Avoid release to the environment. |

## 7. Handling and storage

| | |
|---|---|
| **Precautions for safe handling:** | When using do not eat, drink or smoke. Read and follow manufacturer's recommendations. Use personal protective equipment as required. |
| **Conditions for safe storage, including any incompatibilities:** | Store in a cool, dry place. Keep container tightly closed. |

## 8. Exposure controls/personal protection

**Control Parameters**

**Occupational Exposure Limits**

| Chemical Identity | Type | Exposure Limit Values | Source |
|---|---|---|---|
| Sodium fluoride (NaF) - as F | TWA | 2.5 mg/m3 | US. OSHA Table Z-1-A (29 CFR 1910.1000) (1989) |
| | TWA | 2.5 mg/m3 | US. ACGIH Threshold Limit Values (12 2010) |
| | REL | 2.5 mg/m3 | US. NIOSH: Pocket Guide to Chemical Hazards (2005) |
| | PEL | 2.5 mg/m3 | US. OSHA Table Z-1 Limits for Air Contaminants (29 CFR 1910.1000) (02 2006) |
| Sodium fluoride (NaF) - Dust. | TWA | 2.5 mg/m3 | US. OSHA Table Z-2 (29 CFR 1910.1000) (02 2006) |

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**Biological Limit Values**

| Chemical Identity | Exposure Limit Values | Source |
|---|---|---|
| Sodium fluoride (NaF) (Fluoride: Sampling time: Prior to shift.) | 2 mg/l (Urine) | ACGIH BEI (03 2013) |
| Sodium fluoride (NaF) (Fluoride: Sampling time: End of shift.) | 3 mg/l (Urine) | ACGIH BEI (03 2013) |

**Appropriate Engineering Controls** No special requirements under ordinary conditions of use and with adequate ventilation.

**Individual protection measures, such as personal protective equipment**

**General information:** Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing to remove contaminants. Discard contaminated footwear that cannot be cleaned.

**Eye/face protection:** Wear safety glasses with side shields (or goggles).

**Skin Protection**

**Hand Protection:** Chemical resistant gloves Suitable gloves can be recommended by the glove supplier. Wash hands after contact.

**Other:** Wear a lab coat or similar protective clothing.

**Respiratory Protection:** If engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn.

**Hygiene measures:** Observe good industrial hygiene practices.

## 9. Physical and chemical properties

**Appearance**

**Physical state:** liquid
**Form:** No data available.
**Color:** Clear
**Odor:** Odorless
**Odor threshold:** No data available.
**pH:** 7.0 - 9.0
**Melting point/freezing point:** No data available.
**Initial boiling point and boiling range:** No data available.
**Flash Point:** No data available.
**Evaporation rate:** No data available.

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| | |
|---|---|
| **Flammability (solid, gas):** | No data available. |
| **Upper/lower limit on flammability or explosive limits** | |
| **Flammability limit - upper (%):** | No data available. |
| **Flammability limit - lower (%):** | No data available. |
| **Explosive limit - upper (%):** | No data available. |
| **Explosive limit - lower (%):** | No data available. |
| **Vapor pressure:** | No data available. |
| **Vapor density:** | No data available. |
| **Relative density:** | No data available. |
| **Solubility(ies)** | |
| **Solubility in water:** | Soluble |
| **Solubility (other):** | No data available. |
| **Partition coefficient (n-octanol/water):** | No data available. |
| **Auto-ignition temperature:** | No data available. |
| **Decomposition temperature:** | No data available. |
| **Viscosity:** | No data available. |

## 10. Stability and reactivity

| | |
|---|---|
| **Reactivity:** | Stable under normal temperature conditions and recommended use. |
| **Chemical Stability:** | Material is stable under normal conditions. |
| **Possibility of hazardous reactions:** | Not determined. |
| **Conditions to avoid:** | Avoid exposure to high temperatures or direct sunlight. |
| **Incompatible Materials:** | Metals. Water reactive material. |
| **Hazardous Decomposition Products:** | Stable; however, may decompose if heated. |

## 11. Toxicological information

**General information:** No data on possible toxicity effects have been found.

**Information on likely routes of exposure**

| | |
|---|---|
| **Ingestion:** | No harmful effects expected in amounts likely to be ingested by accident. |
| **Inhalation:** | Limited inhalation hazard at normal work temperatures. |
| **Skin Contact:** | Negligible irritation to skin at ambient temperatures. |
| **Eye contact:** | Elevated temperatures or mechanical action may form vapors, mist, or fumes which may be irritating to the eyes, nose, throat, or lungs. |

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**Symptoms related to the physical, chemical and toxicological characteristics**

**Ingestion:** No data available.

**Inhalation:** No data available.

**Skin Contact:** No data available.

**Eye contact:** No data available.

**Information on toxicological effects**

**Acute toxicity (list all possible routes of exposure)**

**Oral**
**Product:** ATEmix: 13,902.44 mg/kg

**Dermal**
**Product:** No data available.

**Inhalation**
**Product:** No data available.

**Repeated dose toxicity**
**Product:** No data available.

**Skin Corrosion/Irritation**
**Product:** No data available.

**Serious Eye Damage/Eye Irritation**
**Product:** No data available.

**Specified substance(s):**
Sodium fluoride (NaF) Possibly Irritating

**Respiratory or Skin Sensitization**
**Product:** No data available.

**Specified substance(s):**
Sodium fluoride (NaF) Skin sensitization:, in vivo (Guinea pig): Non sensitising

**Carcinogenicity**
**Product:** No data available.

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**IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:**
No carcinogenic components identified

**US. National Toxicology Program (NTP) Report on Carcinogens:**
No carcinogenic components identified

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050):**
No carcinogenic components identified

**Germ Cell Mutagenicity**

**In vitro**
**Product:** No data available.

**In vivo**
**Product:** No data available.

**Reproductive toxicity**
**Product:** No data available.

**Specific Target Organ Toxicity - Single Exposure**
**Product:** No data available.

**Specific Target Organ Toxicity - Repeated Exposure**
**Product:** No data available.

**Aspiration Hazard**
**Product:** No data available.

**Other effects:** No data available.

## 12. Ecological information

**Ecotoxicity:**

**Acute hazards to the aquatic environment:**

**Fish**
**Product:** No negative effects on the aquatic environment are known.

**Aquatic Invertebrates**
**Product:** No negative effects on the aquatic environment are known.

**Chronic hazards to the aquatic environment:**

**Fish**
**Product:** No negative effects on the aquatic environment are known.

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**Aquatic Invertebrates**
**Product:** No negative effects on the aquatic environment are known.

**Toxicity to Aquatic Plants**
**Product:** No negative effects on the aquatic environment are known.

**Persistence and Degradability**

**Biodegradation**
**Product:** Expected to be readily biodegradable.

**BOD/COD Ratio**
**Product:** No data available.

**Bioaccumulative potential**
**Bioconcentration Factor (BCF)**
**Product:** No data available.

**Specified substance(s):**
Sodium fluoride (NaF) Bioconcentration Factor (BCF): 30 Aquatic sediment Other, Key study
Bioconcentration Factor (BCF): 7.5 Aquatic sediment Other, Key study
Bioconcentration Factor (BCF): 27 - 62 Aquatic sediment Other, Key study
Bioconcentration Factor (BCF): 53 - 58 Aquatic sediment Other, Key study
Bioconcentration Factor (BCF): < 2 Aquatic sediment Other, Key study

**Partition Coefficient n-octanol / water (log Kow)**
**Product:** No data available.

**Mobility in soil:** No data available.

**Known or predicted distribution to environmental compartments**
Sodium fluoride (NaF) No data available.

**Other adverse effects:** The product is not expected to be hazardous to the environment.

## 13. Disposal considerations

**General information:** Dispose of waste and residues in accordance with local authority requirements.

**Disposal instructions:** Dispose of waste at an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

**Contaminated Packaging:** No data available.

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## 14. Transport information

| | |
|---|---|
| **DOT**UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Label(s): | Not regulated. |
| Packing Group: | Not regulated. |
| Marine Pollutant: | Not regulated. |
| Limited quantity | Not regulated. |
| Excepted quantity | Not regulated. |
| Special precautions for user: | Not regulated. |

**IMDG**

| | |
|---|---|
| UN Number: | Not regulated. |
| UN Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es) | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| EmS No.: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine Pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

**IATA**

| | |
|---|---|
| UN Number: | Not regulated. |
| Proper Shipping Name: | Not regulated. |
| Transport Hazard Class(es): | |
| Class: | Not regulated. |
| Subsidiary risk: | Not regulated. |
| Packing Group: | Not regulated. |
| Environmental Hazards | |
| Marine pollutant: | Not regulated. |
| Special precautions for user: | Not regulated. |

## 15. Regulatory information

**US Federal Regulations**

**TSCA Section 12(b) Export Notification (40 CFR 707, Subpt. D)**
None present or none present in regulated quantities.

**US. OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)**
None present or none present in regulated quantities.

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**CERCLA Hazardous Substance List (40 CFR 302.4):**

| Chemical Identity | Reportable quantity |
|---|---|
| Sodium fluoride (NaF) | 1000 lbs. |

**Superfund Amendments and Reauthorization Act of 1986 (SARA)**

**Hazard categories**
Not classified
Not classified

**SARA 302 Extremely Hazardous Substance**
None present or none present in regulated quantities.

**SARA 304 Emergency Release Notification**

| Chemical Identity | Reportable quantity |
|---|---|
| Sodium fluoride (NaF) | 1000 lbs. |

**SARA 311/312 Hazardous Chemical**

| Chemical Identity | Threshold Planning Quantity |
|---|---|
| Sodium fluoride (NaF) | 10000 lbs |

**SARA 313 (TRI Reporting)**
None present or none present in regulated quantities.

**Clean Water Act Section 311 Hazardous Substances (40 CFR 117.3)**

| Chemical Identity | Reportable quantity |
|---|---|
| Sodium fluoride (NaF) | Reportable quantity: 1000 lbs. |

**Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130):**
None present or none present in regulated quantities.

**US State Regulations**

**US. California Proposition 65**
No ingredient requiring a warning under CA Prop 65.

**US. New Jersey Worker and Community Right-to-Know Act**
No ingredient regulated by NJ Right-to-Know Law present.

**US. Massachusetts RTK - Substance List**
No ingredient regulated by MA Right-to-Know Law present.

**US. Pennsylvania RTK - Hazardous Substances**
No ingredient regulated by PA Right-to-Know Law present.

**US. Rhode Island RTK**
No ingredient regulated by RI Right-to-Know Law present.

**Becton, Dickinson and Company**
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

## 16.Other information, including date of preparation or last revision

| | |
|---|---|
| **Issue Date:** | 03/29/2019 |
| **Version #:** | 1.1 |
| **Revision Information:** | |
| **Source of information:** | European Chemicals Agency (ECHA): Information on Chemicals. |
| **Further Information:** | No data available. |
| **Disclaimer:** | Disclaimer:<br>The information contained herein has been obtained from various sources and is believed to be correct as of the date issued. However, neither BD nor any of its subsidiaries assumes any liabilities whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability for a particular use of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist. BD provides SDS in electronic form so the information may be more easily accessed. Due to the possibility of errors during transmission, BD makes no representations as to the completeness or accuracy of the information. |

# BD Multitest™ CD3/CD16+CD56/CD45/CD19

50 Tests—Catalog No. 342416

50 Tests with
BD Trucount™ Tubes—Catalog No. 342446

23-5345(11)
2023-07
English

CE 2797 IVD

## 1. INTENDED USE

BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagent with optional BD Trucount™ Tubes is a four-color direct immunofluorescence reagent for use in identifying and determining the percentages and absolute counts of T, B, and natural killer (NK) cells in peripheral blood on a BD flow cytometer equipped with the following:

- At least a 488-nm blue laser and a 640-nm red laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least 4-color fluorescence
- Software to acquire and analyze the data

### Clinical Applications

Determining percentages or absolute counts of $CD3^+$ T lymphocytes or $CD19^+$ B lymphocytes is used to characterize or monitor some forms of immune deficiency and autoimmune diseases.[1,2]

Determining percentages or absolute counts of $CD3^-$ and $CD16^+$ and/or $CD56^+$ NK lymphocytes is used in immunological assessment of hematologically-normal subjects or patients having, or suspected of having, immune deficiency or other immune-mediated diseases.[1,3]

## 2. SUMMARY OF THE TEST

Human peripheral blood contains three types of lymphocytes: T, B, and NK lymphocytes. They have distinct biologic functions and can be identified by differences in their cell-surface antigen expression.

Subsets of antigen-specific T and B lymphocytes have different roles in the adaptive immune response. Helper/inducer T lymphocytes secrete cytokines that help regulate the activity of other T lymphocytes as well as B lymphocytes. Suppressor/cytotoxic T lymphocytes suppress the activity of other T lymphocytes, or recognize and lyse infected or abnormal cells. Antigen-specific B lymphocytes produce and secrete immunoglobulins to regulate the humoral immune response. NK lymphocytes mediate antigen-nonspecific cytotoxicity against infected or abnormal cells.[4]

BD Multitest™ CD3/CD16+CD56/CD45/CD19 with or without BD Trucount™ Tubes is a quantitative assay intended for use by laboratory professionals to identify and enumerate the T-, B-, and NK-lymphocyte subset populations:

- $CD3^+$ T lymphocytes
- $CD19^+$ B lymphocytes
- $CD3^-CD16^+CD56^+$ NK lymphocytes

Automated sample preparation and acquisition can be achieved using the BD FACSDuet™ Sample Preparation System and BD loaders, respectively. Data analysis can be performed using a pre-defined template and automated gating, which can be manually adjusted by the user, if needed.

## Principle of Operation

The BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagent is composed of five monoclonal antibodies, each conjugated to a specific fluorochrome. The reagent is added to peripheral blood and incubated, allowing each monoclonal antibody in the reagent to bind to a specific antigen on the surface of the cells. After incubation, BD FACS™ Lysing Solution is added to lyse the red blood cells in the sample. Cells are acquired on a BD flow cytometer using the appropriate software. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Multitest™ reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. The software and the BD Multitest™ 4-Color assay module are used to analyze the data and report the result.

When determining absolute cell counts, expressed as the number of cells/µL, a precise volume of specimen and BD Multitest™ CD3/CD16+CD56/CD45/CD19 is added to a BD Trucount™ Tube. The BD Trucount™ Tube contains a lyophilized pellet of fluorescent beads. During incubation of the reagent and the specimen, the bead pellet dissolves, releasing a known number of fluorescent beads, which are distinguished from cells by their fluorescence intensity. After lysing red blood cells, the sample is acquired on a BD flow cytometer. The software determines the absolute cell counts by comparing cellular events to bead events, and reports the absolute cell counts in the lab report.

For flow cytometer principles of operation, see the instructions for use (IFU) for your instrument.

# 3. REAGENT

## Reagent Composition

The reagent contains the following conjugated antibodies:

**Table 1** Reagent composition

| Antibody | Fluorochrome | Clone | Isotype | Concentration (µg/mL) |
|---|---|---|---|---|
| CD3 | FITC | SK7[5,6] | $IgG_1\kappa$ | 2.3 |
| CD16 | PE | B73.1[7] | $IgG_1\kappa$ | 1.65 |
| CD56 | PE | NCAM16.2[8] | $IgG_{2b}\kappa$ | 1.1 |
| CD45 | PerCP | 2D1[9] | $IgG_1\kappa$ | 7.50 |
| CD19 | APC | SJ25C1[10] | $IgG_1\kappa$ | 2.3 |

CD3 (SK7) recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[11] The CD3 antigen is present on T lymphocytes and is noncovalently associated with either α/β or γ/δ TCR.[12] CD3 reacts minimally with other cell populations.[13]

CD16 (B73.1) and CD56 (NCAM16.2) together facilitate identification of the NK-lymphocyte population.[14,15]

- CD16 (B73.1) recognizes a human NK-lymphocyte antigen that is an Fc receptor for IgG.[16,17,18] CD16 also reacts with neutrophils[19] and with granulocytes to a variable extent.[16]

- CD56 (NCAM16.2) recognizes an extracellular immunoglobulin-like domain of the neural cell adhesion molecule (NCAM).[20,21,22] CD56 also reacts with approximately 5% of $CD3^+$ peripheral blood lymphocytes.[19]

CD45 (2D1) recognizes all isoforms of the leucocyte common antigen (LCA)/T200 family.[23] The CD45 antigen is present on all human leucocytes, including lymphocytes, monocytes, granulocytes, eosinophils, and basophils in peripheral blood.[23] CD45 has been reported to react weakly with mature circulating erythrocytes and platelets.[23,24]

CD19 (SJ25C1) recognizes an antigen that is present on human B lymphocytes at all stages of maturation,[10,25,26] but is lost on plasma cells.[26] CD19 does not react with resting or activated T lymphocytes, granulocytes, or monocytes.[27]

### Precautions

- The reagent should be clear. Do not use the reagent if you observe any change in appearance. Precipitation, cloudiness, or change in color indicates instability or deterioration.
- The antibody reagent contains sodium azide as a preservative. However, take care to avoid microbial contamination, which can cause erroneous results.
- If using BD Trucount™ Tubes, calibrate pipets to deliver exactly 50 µL of sample or perform the reverse pipetting technique (see Reverse Pipetting on page 7). See the pipet manufacturer's instructions for more information.
- Bead count varies by lot of BD Trucount™ Tubes. It is critical to use the bead count shown on the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of BD Trucount™ Tubes in the same run.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

### Storage and Handling

- Store the reagent at 2–8 °C. Reagent in opened or unopened vials is stable until the expiration date shown on the vial label. Do not use after this expiration date.
- Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the reagent vial dry.
- The reagent is stable if kept in the BD FACSDuet™ instrument for 8 hours per day for 5 days. Do not store the reagent overnight in the instrument. Use of any reagent remaining after being kept in the BD FACSDuet™ instrument for 5 days must be validated by the user.
- Store BD Trucount™ Tubes in their original foil pouch at 2–25 °C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. Do not remove the desiccant pack from the pouch. Use tubes within 1 hour after removal from the foil pouch.
- BD Trucount™ Tubes in an unopened pouch are stable until the expiration date shown on the packaging. Do not use tubes after the expiration date.
- Tubes in an opened pouch are stable for 1 month after the date of opening, when stored as directed. Write the date when you first open the pouch in the space provided on the label.

## 4. INSTRUMENT

The BD FACSLyric™ and BD FACSCanto™ II systems are outlined in the following table. See the corresponding reagent or instrument user documentation for details.

**Table 2** BD FACSLyric™ and BD FACSCanto™ II systems

| Flow cytometer | Setup beads | Setup software | Analysis software | Assay module |
|---|---|---|---|---|
| BD FACSLyric™ | BD® CS&T Beads[a]<br>BD® FC Beads 7-Color Kit[b] | BD FACSuite™ Clinical application | BD FACSuite™ Clinical application | BD Multitest™ 4-Color |
| BD FACSCanto™ II | BD FACS™ 7-Color Setup Beads[c] | BD FACSCanto™ Clinical Software v2.4 or later | BD FACSCanto™ Clinical Software v2.4 or later | BD Multitest™ 4-Color |

a. To perform daily cytometer quality control.

b. To calculate compensation.

c. To set photomultiplier tube (PMT) voltages and fluorescence compensation, and check instrument sensitivity before use.

The BD FACS™ Loader and BD FACS™ Universal Loader can be used with this product. See the IFU for the cytometer used with your Loader for more information.

The BD FACSDuet™ sample preparation system can be used with this product. See the *BD FACSDuet™ Sample Preparation System Instructions for Use* for more information.

## 5. SPECIMEN COLLECTION AND PREPARATION

- Collect blood specimens aseptically by venipuncture into a BD Vacutainer® EDTA blood collection tube, or equivalent.[28]

  BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes has been validated with both liquid and dry formulations of EDTA. The reagent has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount™ Tubes.

  The assay requires 50 µL of peripheral blood per test. We recommend starting with a minimum of 100 µL of blood to accommodate the excess volume needed to perform reverse pipetting.
- If using the dual platform method, obtain a white blood cell (WBC) count and a differential white cell count from the same whole blood sample before staining to calculate absolute counts from percentages. See Dual Platform Method on page 14.
- Store blood specimens at room temperature (20–25 °C).
- Stain specimens within 48 hours of draw.
- Acquire samples within 24 hours of staining.

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[29,30] 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.[31]

### Interference

Substances present in the specimen might interfere with the assay:

- Specimens obtained from patients taking immunosuppressive drugs[32,33,34] or undergoing monoclonal antibody treatment[35,36,37,38,39,40] can yield erroneous results.
- Hemolyzed samples can interfere with the assay and should be rejected.[41] Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated before staining can give aberrant results.
- Blast cells can interfere with test results.[42]
- Lipemic specimens can interfere with the assay.[43,44]
- Bilirubin interferes at an absorbance peak of 456 nm.[45]

## Interfering Conditions

The following table lists the substances that were tested for interference with a similar reagent, the BD Multitest™ 6-Color TBNK reagent with optional BD Trucount™ Tubes.

Testing for interference was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[46] There was no detectable interference at the following concentrations.

**Table 3** Non-interfering substances

| Analyte | Concentration tested |
|---|---|
| Acetaminophen | 156 µg/mL |
| Acetylsalicylic acid (Aspirin) | 30 µg/mL |
| Albuterol | 0.015 µg/mL |
| Atenolol | 3 µg/mL |
| Atorvastatin | 0.25 µg/mL |
| Azithromycin | 3.7 µg/mL |
| Bilirubin, conjugated | 2 mg/dL |
| Cobicistat | 3.6 µg/mL |
| Efavirenz | 12 µg/mL |
| Enoxaparin | 2 µg/mL |
| Guaifenesin | 1.5 µg/mL |
| Hydroxychloroquine | 0.2 µg/mL |
| Ibuprofen | 73 µg/mL |
| Insulin | 37 µU/mL |
| Kaletra | 15.5 µg/mL |
| Lisinopril | 0.082 µg/mL |
| Maraviroc | 0.888 µg/mL |
| Oseltamivir | 0.133 µg/mL |
| Raltegravir | 15 µg/mL |
| Remdesivir | 16.32 µg/mL |
| Ritonavir | 15 µg/mL |
| Tenofovir | 0.978 µg/mL |
| Tocilizumab | 149.4 µg/mL |
| Vancomycin | 40 µg/mL |

The following substances interfered with the assay at the indicated concentration:

**Table 4** Interfering substances

| Analyte | Concentration tested |
|---|---|
| Albumin[a,e] | 6 g/dL |
| Bilirubin, unconjugated[b,e] | 2 mg/dL |
| Erythrocytes[c,e] | $6x10^3$ cells/µL |
| Hemoglobin[c,e] | 1000 mg/dL |
| Triglycerides[d,e] | 1500 mg/dL |

a. Albumin interferes as a result of its comparatively large concentration in the peripheral blood and its ability to bind as well as to release large quantities of ligands.47

b. Unconjugated Bilirubin may induce autofluorescence.48

c. The presence of red blood cells (RBCs) in the sample preparation can cause light interference and non-specific interactions leading to erroneous test results.49 Hemolyzed samples should be rejected. The hemoglobin concentration refers to free hemoglobin.

d. Immunomodulatory drugs used for treatment of HIV infection may cause lipemia. Lipemia is known to interfere in assays that use the transmission of light and impact the scattering of light.50,51

e. The listed endogenous substances interfere with the assay at higher than normal concentrations, i.e. hyperalbuminemia, unconjugated hyperbilirubinemia, erythrocytosis, hemoglobinemia, and hypertriglyceridemia. Interference caused by these endogenous substances is not uncommon and has been described in the literature (see references listed in notes a–d).

# 6. PROCEDURE

## Reagents and Materials

## Reagents and materials provided

BD Multitest™ CD3/CD16+CD56/CD45/CD19 is provided in 1 mL of buffered saline with <0.1% sodium azide. The reagent is sufficient for 50 tests.

If calculating absolute counts, use BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes. The reagent comes with two pouches of BD Trucount™ Tubes. Each pouch contains 25 tubes, sufficient for 25 tests. The tubes contain a freeze-dried pellet of fluorescent beads in a single-use tube.

## Reagents and materials required but not provided

- BD FACS™ Lysing Solution (Catalog No. 349202)

  The lysing solution is provided as a 10X concentrate and it contains diethylene glycol and formaldehyde. See the *BD FACS™ Lysing Solution* IFU for precautions and warnings.
- Disposable 12 × 75-mm capped polystyrene test tubes, or equivalent (if not using BD Trucount™ Tubes)
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing 1X BD FACS™ Lysing Solution
- BD Multi-Check™ Control (Catalog Nos. 340911, 340912, 340913)
- BD Multi-Check™ CD4 Low Control (Catalog Nos. 340914, 340915, 340916)
- (Optional) BD Trucount™ Controls (Catalog No. 340335)
- (Optional) BD FACS™ Universal Loader
- (Optional) BD FACS™ Loader (used on the BD FACSCanto™ II flow cytometer)

## 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

Accurate pipetting is critical when using a BD Trucount™ Tube. Use the reverse pipetting technique to add the sample to a BD Trucount™ Tube. 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.

## Performing Quality Control

Run two levels of process control material (for example, BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control) before acquiring patient specimens.[52] Control materials should provide established values for percent positive and absolute counts for the relevant cell populations. Process the controls like patient specimens to monitor the performance of the entire analytic process. This is done at least once each day when patient testing is performed.

**NOTE** BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control are validated as process controls on BD FACSLyric™ flow cytometers.

If needed, use BD Trucount™ Controls to verify pipetting accuracy and the bead count value of the BD Trucount™ Tubes.

## Staining the Cells

If using the BD FACSDuet™ system to prepare the samples, see the *BD FACSDuet™ Sample Preparation System Instructions for Use*.

1. For each sample, remove a tube and label it with the appropriate reagent and sample identification.

   For calculating absolute counts and lymphocyte subset percentages, label a BD Trucount™ Tube. For calculating lymphocyte subset percentages only, label a 12 × 75-mm tube.

   **NOTE** For samples stained in BD Trucount™ Tubes, verify that the BD Trucount™ bead pellet is under 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. Do not transfer beads to another tube.
2. Pipette 20 µL of the appropriate BD Multitest™ reagent into the bottom of the tube.

   If using a BD Trucount™ Tube, pipette the reagent onto the side of the tube, just above the metal retainer, without touching the bead pellet.
3. Pipette 50 µL of well-mixed control material or anticoagulated peripheral blood onto the side of the tube.

   If using a BD Trucount™ Tube, pipette the sample onto the side of the tube, just above the metal retainer, without touching the bead pellet.

   **NOTE** Thoroughly mix the controls before pipetting them. See the *BD Multi-Check™ Control* or *BD Multi-Check™ CD4 Low Control* IFU for detailed instructions.

   **NOTE** Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer. See Reverse Pipetting on page 7. Avoid smearing sample down the side of the tube. If whole blood or control material remains on the side of the tube, it will not be stained with the reagent and can affect results.
4. Cap the tube and vortex gently to mix.
5. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).
6. Add 450 µL of 1X BD FACS™ Lysing Solution to the tube.
7. Cap the tube and vortex gently to mix.
8. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).

   The sample is now ready to be analyzed on the flow cytometer. Acquire the sample within 24 hours of staining. Store the stained sample in the dark at room temperature (20–25 °C) until acquisition.

## Running the Assay on a BD FACSLyric™ Flow Cytometer

### Before you begin:

1. Ensure that Characterization QC (CQC) and lyse/no wash reference settings have not expired.
2. Add reagent lots to library, if needed.

   See the *BD FACSLyric™ System Instructions For Use* for information.
3. Perform daily Performance QC (PQC) using BD® CS&T Beads.

   See the *BD® CS&T Beads* IFU and the *BD FACSLyric™ System Instructions For Use* for information.

### To run the assay:

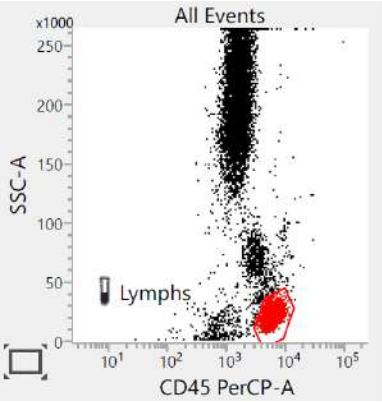
1. Create a worklist.
   - Create a Multi-Check™ Control task for each process control you are running.
   - Create an appropriate assay task for each patient specimen you are running.
2. Enter information in the worklist table.
   - If not using BD Trucount™ Tubes, enter the WBC count and the percentage of lymphocytes (WBC (x1000) and Lymphs (%), respectively), or the lymphocyte count (Lymphs (x1000)) in the appropriate column.

   **NOTE** Divide the WBC count or the lymphocyte count by 1,000 before entering it into the software.
   - If using BD Trucount™ Tubes, enter the lot ID for the tubes and the bead count, found on the pouch label, in the appropriate column (Trucount Lot ID and Beads Per Pellet, respectively).
3. Run the control tasks on the worklist.
4. Vortex each tube thoroughly at low speed immediately before acquiring it.[53]

   **NOTE** If you are using the BD FACS™ Universal Loader, vortex tubes immediately before placing them into the Loader racks.
5. After acquiring the control samples, click **Stop Tube**.

   **NOTE** This assumes that process control passes. Stop it to verify, then continue with samples of interest. If process control fails, restain samples and process controls because you cannot discriminate whether process control failure comes from staining or the instrument.
6. Review the lab report for the controls.
7. Visually inspect the CD45 PerCP-A vs SSC-A dot plot.

   The lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes should also appear as distinct clusters. Do not proceed with analysis if populations are diffuse and there is little or no separation between clusters.

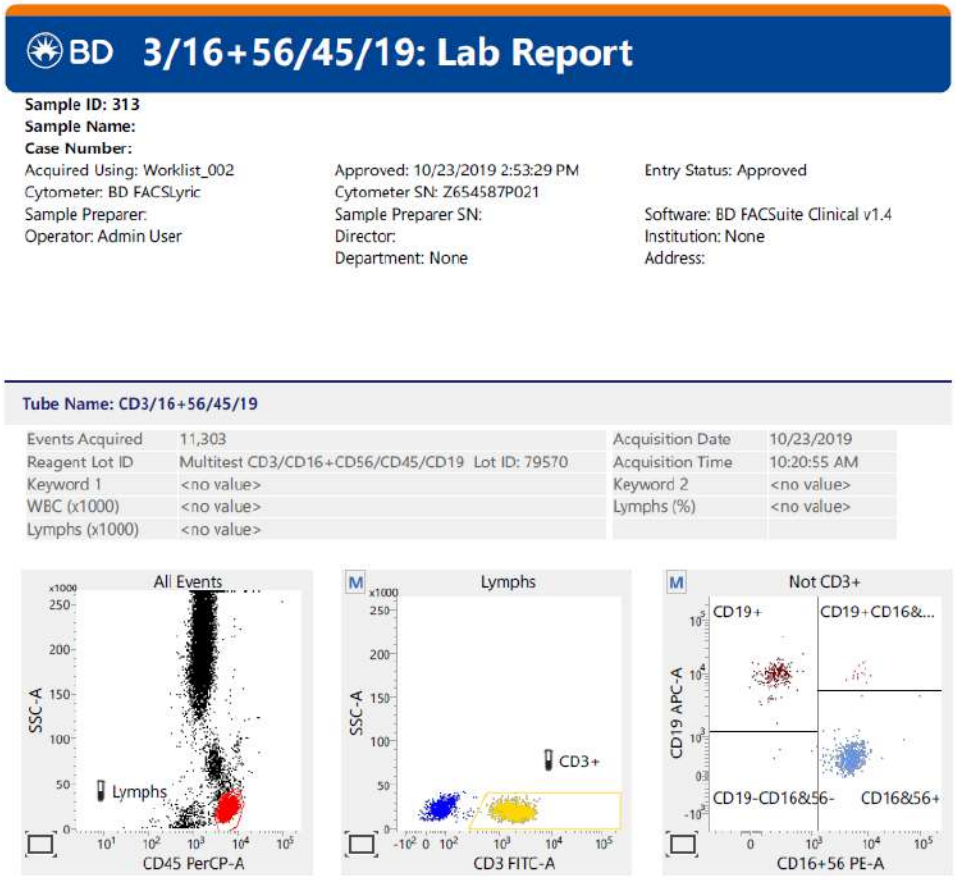



8. Verify that the results are within the values reported in the Assay Values sheet, provided with the controls.
9. Set the run pointer to the first patient specimen and select **Run from Pointer** from the **Run** menu.

Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.

10. Review the assay lab report.

    Page 1 of the lab report shows dot plots to identify the cell populations. The lab report shown is for BD Multitest™ CD3/CD16+CD56/CD45/CD19 without BD Trucount™ Tubes.



BD 3/16+56/45/19: Lab Report

**Sample ID: 313**
**Sample Name:**
**Case Number:**
Acquired Using: Worklist_002
Cytometer: BD FACSLyric
Sample Preparer:
Operator: Admin User

Approved: 10/23/2019 2:53:29 PM
Cytometer SN: Z654587P021
Sample Preparer SN:
Director:
Department: None

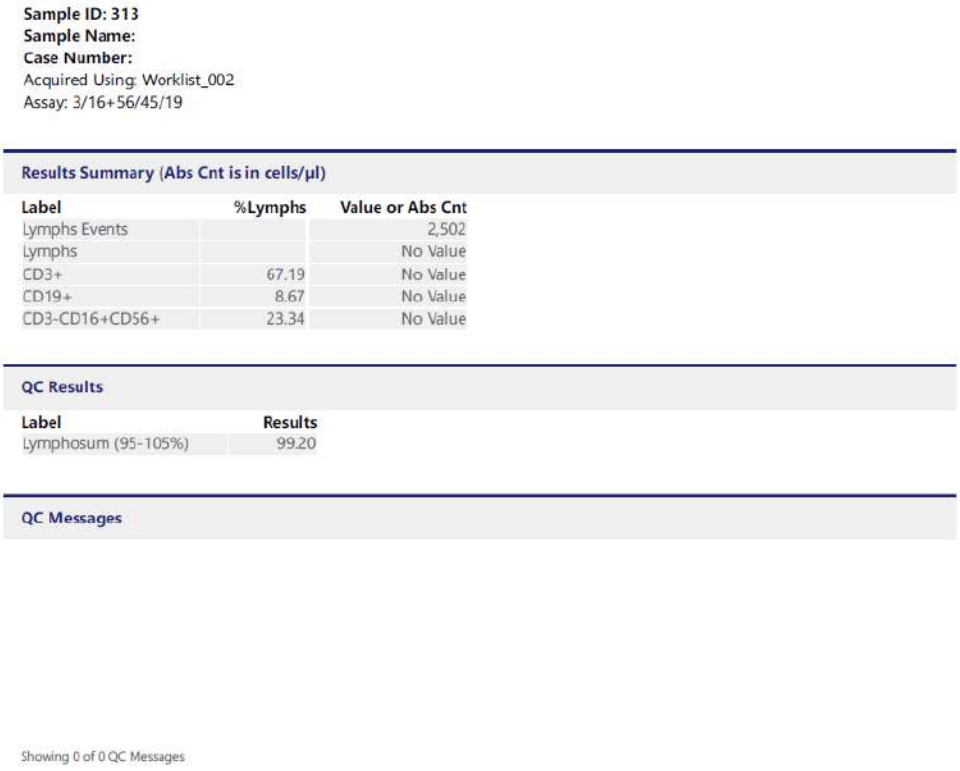
Entry Status: Approved

Software: BD FACSuite Clinical v1.4
Institution: None
Address:

**Tube Name: CD3/16+56/45/19**

| | | | |
|---|---|---|---|
| Events Acquired | 11,303 | Acquisition Date | 10/23/2019 |
| Reagent Lot ID | Multitest CD3/CD16+CD56/CD45/CD19 Lot ID: 79570 | Acquisition Time | 10:20:55 AM |
| Keyword 1 | <no value> | Keyword 2 | <no value> |
| WBC (x1000) | <no value> | Lymphs (%) | <no value> |
| Lymphs (x1000) | <no value> | | |

Page 2 of the lab report summarizes the results, presents QC results for the assay, and presents any QC messages that were triggered.



**Sample ID: 313**
**Sample Name:**
**Case Number:**
Acquired Using: Worklist_002
Assay: 3/16+56/45/19

**Results Summary (Abs Cnt is in cells/µl)**

| Label | %Lymphs | Value or Abs Cnt |
|---|---|---|
| Lymphs Events | | 2,502 |
| Lymphs | | No Value |
| CD3+ | 67.19 | No Value |
| CD19+ | 8.67 | No Value |
| CD3-CD16+CD56+ | 23.34 | No Value |

**QC Results**

| Label | Results |
|---|---|
| Lymphosum (95-105%) | 99.20 |

**QC Messages**

Showing 0 of 0 QC Messages

See the *BD FACSLyric™ System Instructions for Use* or the *BD FACSLyric™ Clinical Reference System* for more information.

## Running the Panel on a BD FACSCanto™ II Flow Cytometer

1. Run Setup using BD FACS™ 7-Color Setup Beads.
   See the *BD FACSCanto™ II Instructions for Use* for more information.
2. Add a BD Multitest™ CD3/CD16+CD56/CD45/CD19 panel entry for each process control and patient sample.

   **NOTE** The word "Control" must appear in the sample name of the process controls.
3. Acquire the process control samples.
4. Vortex each tube thoroughly at low speed immediately before acquiring it. It is important to reduce aggregation before running samples on the flow cytometer.

   **NOTE** If you are using the BD FACS™ Loader, vortex tubes immediately before placing them into the Loader racks.
5. Verify that the process control values are within the manufacturer's expected ranges.
6. Acquire the patient samples.
7. Review the assay lab report.

The lab report shows dot plots to identify the cell populations. It also shows a table containing results for individual populations, QC results related to the data, and any QC messages that were triggered. The lab report shown is for BD Multitest™ CD3/CD16+CD56/CD45/CD19 without BD Trucount™ Tubes.

Do7280i

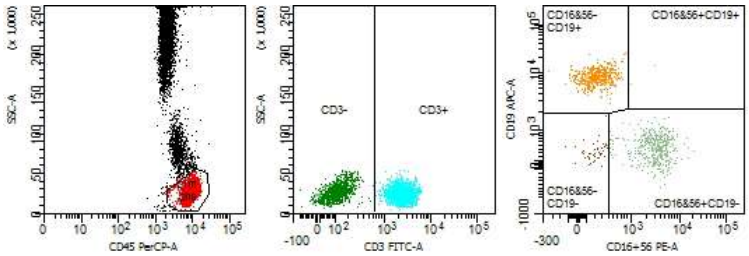
Director:

| | |
|---|---|
| Panel: | 3/16+56/45/19 |
| Acquired: | 10/28/2015 3:43:29 PM |
| Analyzed: | 10/28/2015 3:43:29 PM |
| Status: | OK |
| Operator: | Lab Manager |
| Reviewer: | 28102015.csv |
| Results: | |

WBC Count (x1000): 5 Lymphs (%): 10 Lymphs (x1000):

BD FACSCanto II V96300004 BD FACSCanto v.3.1.5773.39345

CD3/CD16+56/CD45/CD19 Total Events: 10058




Do7280i027.001.fcs Reagent Lot ID: 00000

| Parameter | Percent | Value/AbsCnt |
|---|---|---|
| Lymph Events | | 3318 |
| CD3+ | 70.83 | 354.13 |
| CD16+CD56+ | 13.20 | 66.00 |
| CD19+ | 14.59 | 72.94 |
| CD45+ | | 500 |

QC Messages

Lymphosum is: 98.61

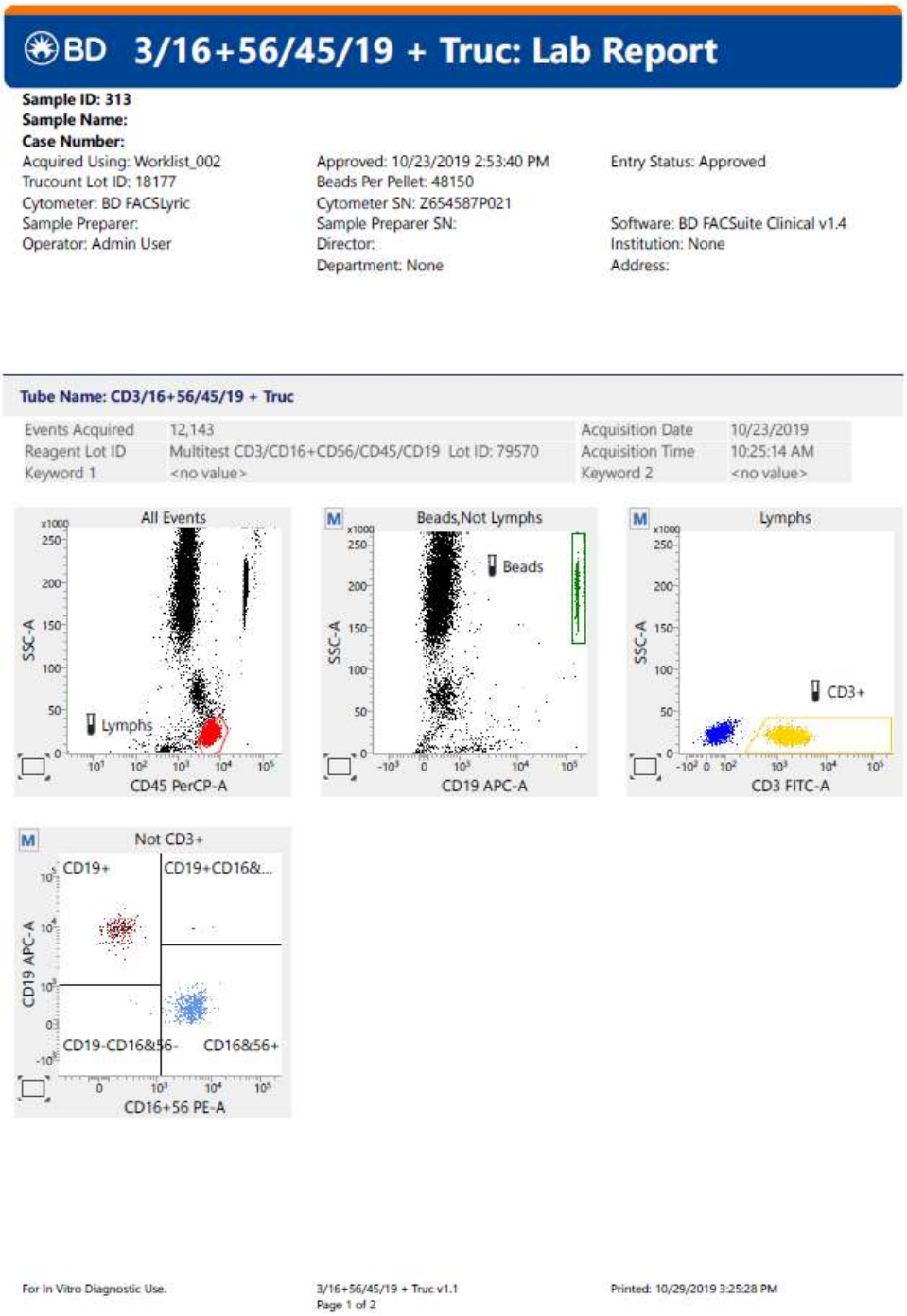
Comments

# 7. RESULTS

## Representative Data

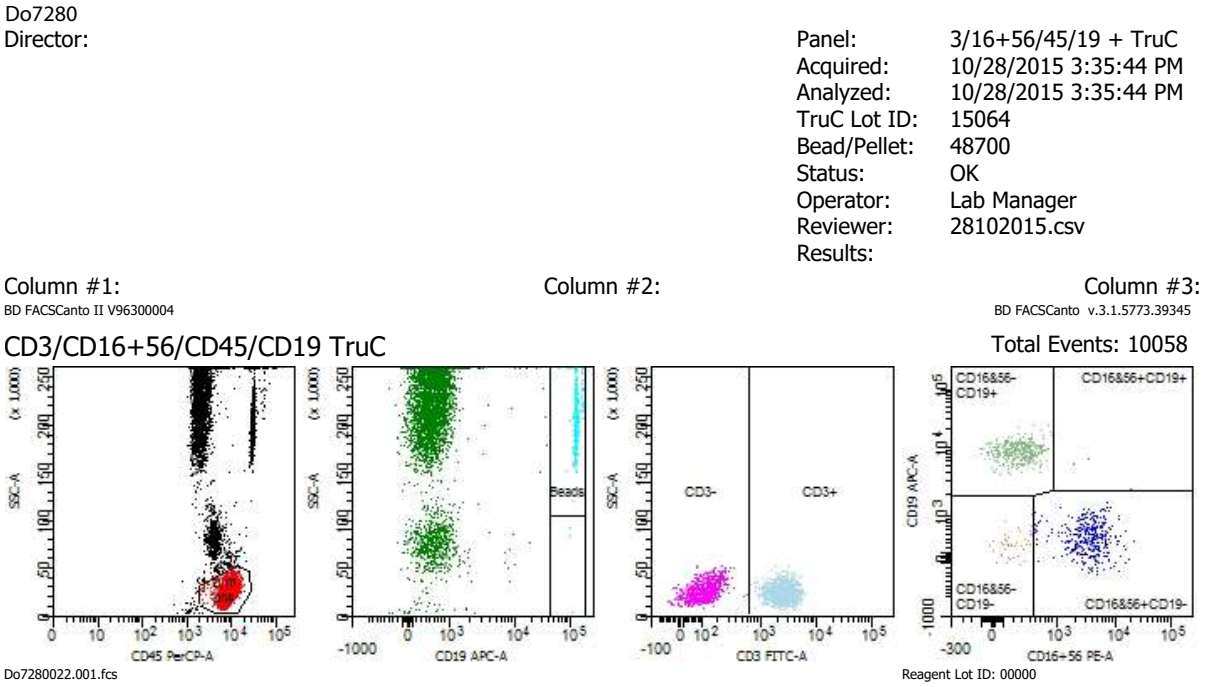
A hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube was acquired on a BD FACSLyric™ flow cytometer. See Figure 1.

**Figure 1** BD FACSLyric™ laboratory report showing data collected with BD Trucount™ Tubes.



A similar sample was acquired on a BD FACSCanto™ II flow cytometer.

**Figure 2** BD FACSCanto™ II laboratory report showing data collected with BD Trucount™ Tubes.




The lymphocyte subsets are identified using the following gating strategy:

**Table 5** Gating strategy for BD Multitest™ CD3/CD16+CD56/CD45/CD19

| Dot plot | Parent population | Gate | Populations identified |
|---|---|---|---|
| CD45 PerCP-A vs SSC-A | All Events | Lymphs | Lymphocytes |
| CD19 APC-A vs SSC-A | Beads, Not Lymphs | Beads | Trucount beads |
| CD3 FITC-A vs SSC-A | Lymphs | $CD3^+$ | $CD3^+$ T lymphocytes |
| CD16+56 PE-A vs CD19 APC-A | Not $CD3^+$ ($CD3^-$) | Quadrant | $CD19^+$<br>$CD19^+CD16\&56^+$<br>$CD16\&56^+$<br>$CD19^-CD16\&56^-$ |

The second dot plot, used to identify Trucount™ beads, is present in the 3/16+56/45/19 + Truc Lab Report only.

For information about gating and troubleshooting, see the instructions for use for your flow cytometer.

## Calculating Absolute Counts

When using cytometer-specific BD software, results show positive cells as a percentage of lymphocytes. In addition, the software uses one of two methods to calculate absolute counts of positive cells per microliter of blood (cells/µL).

## Single Platform Method

When BD Trucount™ Tubes are used, the absolute number of positive cells in the sample can be determined by comparing cellular events to bead events. The software calculates absolute counts using the following formula:

$$\frac{\text{\# events in cell population}}{\text{\# events in absolute count bead region}} \times \frac{\text{\# beads/test}}{\text{test volume}} = \text{cell population absolute count}$$

The # beads/test is found on the BD Trucount™ Tubes foil pouch label and varies from lot to lot.

## Dual Platform Method

This method is used when using 12 × 75-mm polystyrene tubes (or equivalent) instead of BD Trucount™ Tubes. When creating the worklist, enter values for either the lymphocyte count, or the WBC count and the percentage of lymphocytes, as determined by a hematology analyzer or another method. See the instructions for use for your instrument for more information. The software uses one of the following formulas to calculate absolute counts:

- User provides lymphocyte count per µL.

$$\frac{\text{\# events in cell population} \times \text{lymphocyte count per µL}}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

- User provides WBC count per µL and percentage of lymphocytes.

$$\frac{\text{\# events in cell population} \times \text{WBC count} \times (\%\text{lymphocytes}/100)}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

**NOTE** The accuracy of the absolute counts determined with the Dual Platform Method depends upon the accuracy of the values entered into the software.

# 8. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the lymphocyte subsets identified using BD Multitest™ CD3/CD16+CD56/CD45/CD19. Age, gender, clinical characteristics, and ethnicity of patients should be known when a reference interval is determined.[54] The provided reference intervals are for information only.
- BD Multitest™ CD3/CD16+CD56/CD45/CD19 is not intended for screening samples for the presence of leukemic cells or for immunophenotyping samples from leukemia patients.
- Absolute counts are not comparable between laboratories using different manufacturers' equipment.
- BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants to determine absolute counts.

# 9. REFERENCE INTERVALS

Reference intervals for BD Multitest™ CD3/CD16+CD56/CD45/CD19 with and without BD Trucount™ Tubes were determined in a study using the BD FACSLyric™ flow cytometer.[48] The study objective was to establish device reference interval values in stained peripheral blood from a healthy cohort of male and female adults

that are free of hematological abnormality. Device reference interval refers to a specified interval of the distribution of lymphocyte subset absolute count and percent values taken from a biological reference population. Blood from a population of healthy control subjects was stained with the BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes, and then acquired and analyzed on a BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application. See the first limitation (in the preceding section) for more information about reference intervals.

**Table 6** Representative reference intervals for BD Multitest™ CD3/CD16+CD56/CD45/CD19

| Lymphocyte subset | N[a] | Units | Mean | 95% range |
|---|---|---|---|---|
| $CD3^+$ | 130 | % | 71.77 | 56.74–82.54 |
| | | cells/µL | 1,560.44 | 812–2,655 |
| $CD19^+$ | 130 | % | 13.69 | 5.14–22.96 |
| | | cells/µL | 292.73 | 60–551 |
| $CD3^-CD16^+CD56^+$ | 130 | % | 13.25 | 5.42–29.65 |
| | | cells/µL | 281.04 | 102–617 |

a. N = number of samples

# 10. PERFORMANCE CHARACTERISTICS

## Specimen Handling and Collection (AOB/AOS)

A study was performed to assess the age of blood (AOB) and age of stain (AOS) using BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes. The stability of EDTA-anticoagulated blood was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring stained sample

Peripheral blood specimens were tested to at least 51 hours post draw and stained samples were tested to at least 26 hours post stain. All samples were maintained at room temperature (20–25 °C) before staining or acquisition.

Based on the results of this study, we recommend staining samples within 48 hours of draw and analyzing samples within 24 hours of staining.

## Limit of Blank and Limit of Detection

The detection capability of the BD Multitest™CD3/CD16+CD56/CD45/CD19 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. Limit of Blank (LOB) refers to the highest apparent absolute count values that can be detected in a stained sample containing no lymphocytes. Limit of Detection (LOD) refers to the lowest absolute count values that can be detected above zero in a stained sample containing a very low $CD3^+CD4^+$ lymphocyte concentration.

Cell-free plasma samples were used to estimate LOB. Plasma samples containing 10 ±5 $CD3^+CD4^+$ cells/µL were used to estimate LOD. Sixty replicates of each sample type were stained manually or using the BD FACSDuet™ system with each of three reagent lots.

Three BD FACSLyric™ flow cytometers were used to acquire the manually prepared samples. A minimum of one BD FACSDuet™ system integrated with a BD FACSLyric™ flow cytometer was used in the other study. Absolute count values for LOB and LOD are shown in the following table.

**Table 7** Detection capability of BD Multitest™ CD3/CD16+CD56/CD45/CD19 (LOB and LOD)

| | Manual sample preparation | | Sample preparation with BD FACSDuet™ system | |
|---|---|---|---|---|
| **Lymphocyte subset** | **LOB (cells/µL)** | **LOD (cells/µL)** | **LOB (cells/µL)** | **LOD (cells/µL)** |
| $CD3^+$ | 4 | 9 | 6 | 16 |
| $CD19^+$ | 2 | 5 | 0 | 4 |
| $CD3^-CD16^+CD56^+$ | 1 | 6 | 0 | 7 |

## Limit of Quantitation

The limit of quantitation (LOQ) of the BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. LOQ refers to the lowest lymphocyte absolute count values that can be quantitatively detected with stated accuracy in samples containing a range of very low $CD3^+CD4^+$ concentration. Plasma samples containing 10, 20, 30, or 50 $CD3^+CD4^+$ cells/µL were used to estimate LOQ.

In the study on the BD FACSLyric™ flow cytometer, 40 replicates of samples from each of the four concentration levels were stained using two lots of the BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagents. For the comparator system, 10 of the 40 replicates from each concentration level were stained and acquired on a BD FACSCanto™ II flow cytometer. Three BD FACSLyric™ flow cytometers and one BD FACSCanto™ II flow cytometer were used in the study.

In the study using the BD FACSDuet™ system, 10 replicates from each concentration level were stained with three lots of the reagents using the BD FACSDuet™ system and acquired using an integrated BD FACSLyric™ flow cytometer. For the comparator system, five replicates from each concentration level were stained manually with three lots of the reagents and acquired on a BD FACSLyric™ flow cytometer. Three integrated BD FACSDuet™–BD FACSLyric™ systems and one standalone BD FACSLyric™ flow cytometer were used in the study. Absolute count values for LOQ are shown in the following table.

**Table 8** Detection capability of BD Multitest™ CD3/CD16+CD56/CD45/CD19 (LOQ)

| | Manual sample preparation (first study) | Sample preparation with BD FACSDuet™ system (second study) |
|---|---|---|
| **Lymphocyte subset** | **LOQ (cells/µL)** | **LOQ (cells/µL)** |
| $CD3^+$ | 14 | 19 |
| $CD19^+$ | 14 | 15 |
| $CD3^-CD16^+CD56^+$ | 10 | 13 |

## BD FACSLyric™ Flow Cytometer

### Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer

A study was performed at five sites to demonstrate equivalency between acquisition using the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer. Peripheral blood specimens were collected from normal donors and HIV-infected individuals using BD Vacutainer® EDTA blood collection tubes. Specimens were stained using BD Multitest™ CD3/CD16+CD56/CD45/CD19 in BD Trucount™ Tubes and acquired on a BD FACSLyric™ flow cytometer using the BD FACSuite™ Clinical application. Lymphocyte subset percentages and absolute counts were enumerated. The results were compared with results from the same samples acquired on a BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software.

Method comparison statistics are reported for all cell subsets.[55] See the following table.

**Table 9** Method comparison statistics for lymphocyte subsets

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| $CD3^+$ | 362 | % | 0.99 | 1.00 | 0.51 | 1.38–97.68 |
| | | cells/µL | 0.99 | 1.04 | –0.62 | 6–9,189 |
| $CD19^+$ | 362 | % | 1.00 | 1.02 | –0.18 | 0.00–92.43 |
| | | cells/µL | 0.99 | 1.02 | –0.05 | 0–4,252 |
| $CD3^-CD16^+CD56^+$ | 362 | % | 0.99 | 0.99 | –0.81 | 1.09–87.67 |
| | | cells/µL | 0.99 | 0.96 | –3.79 | 14–2,151 |

### Method comparison, BD FACS™ Universal Loader vs manual acquisition

A study was performed at one site to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Peripheral blood specimens were stained in duplicate using BD Multitest™ CD3/CD16+CD56/CD45/CD19 with BD Trucount™ Tubes. Stained samples were acquired on one of three BD FACSLyric™ flow cytometers using either the BD FACS™ Universal Loader or manual acquisition.

The mean, difference, and relative difference for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for lymphocyte subset percentages and absolute counts. See the following table.

**Table 10** BD FACS™ Universal Loader vs manual acquisition

| Lymphocyte subset | N | Units | Mean | | Difference | Relative difference |
|---|---|---|---|---|---|---|
| | | | Loader | Manual | | |
| $CD3^+$ | 72 | % | 73.97 | 74.01 | –0.03 | –0.05 |
| | | cells/µL | 1,484.40 | 1,511.13 | –26.72 | –1.24 |
| $CD19^+$ | 72 | % | 12.87 | 12.95 | –0.08 | –0.96 |
| | | cells/µL | 250.65 | 255.93 | –5.28 | –1.83 |

| Lymphocyte subset | N | Units | Mean | | Difference | Relative difference |
|---|---|---|---|---|---|---|
| | | | Loader | Manual | | |
| $CD3^-$ $CD16^+CD56^+$ | 72 | % | 12.13 | 12.08 | 0.06 | 0.67 |
| | | cells/µL | 215.79 | 220.47 | –4.68 | –0.42 |

## Method comparison, BD FACSLyric™ with BD FACSDuet™ system vs standalone BD FACSLyric™

Peripheral blood specimens were collected at three clinical study sites. An aliquot of each specimen was stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube using the BD FACSDuet™ system. Stained samples were automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application. A second aliquot of each specimen was stained manually with the reagents in a BD Trucount™ Tube. Stained samples were acquired on a standalone BD FACSLyric™ flow cytometer using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application.

Results were compared between samples prepared using the BD FACSDuet™ system and samples prepared manually. Method comparison statistics are reported for all cell subsets. See the following table.

**Table 11** Method comparison statistics for lymphocyte subsets

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| $CD3^+$ | 373 | % | 0.98 | 1.00 | 0.27 | 44.12–99.07 |
| | | cells/µL | 0.98 | 1.00 | –3.18 | 85–11,613 |
| $CD19^+$ | 373 | % | 0.97 | 1.00 | –0.03 | 0.17–31.8 |
| | | cells/µL | 0.98 | 0.99 | –0.08 | 8–2,236 |
| $CD3^-CD16^+CD56^+$ | 373 | % | 0.98 | 1.00 | 0.23 | 0.52–44.27 |
| | | cells/µL | 0.98 | 1.02 | 0.28 | 9–2,188 |

## Precision (repeatability), control material (standalone BD FACSLyric™ flow cytometer)

A 21-day single-site precision study was performed to assess repeatability and within-site precision using control material.[56] Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across four BD FACSLyric™ flow cytometers and four operators by acquiring two concentrations of analyte, CD-Chex Plus® control (CDN) and CD-Chex Plus® CD4 Low control (CDL), stained in duplicate using four lots of BD Multitest™ CD3/CD16+CD56/CD45/CD19. Two separate runs were analyzed during each of the 21 tested days.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts using control material, respectively.

**Table 12** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 76.73 | 0.80 | 0.84 |
| $CD19^+$ | 12.09 | 0.55 | 0.55 |
| $CD3^-CD16^+CD56^+$ | 10.34 | 0.59 | 0.59 |

**Table 13** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 57.20 | 1.09 | 1.17 |
| $CD19^+$ | 21.70 | 0.79 | 0.82 |
| $CD3^-CD16^+CD56^+$ | 19.40 | 0.84 | 0.85 |

**Table 14** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,738.01 | 4.00 | 4.12 |
| $CD19^+$ | 273.84 | 6.02 | 6.16 |
| $CD3^-CD16^+CD56^+$ | 234.38 | 7.41 | 7.52 |

**Table 15** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 871.97 | 3.82 | 3.97 |
| $CD19^+$ | 330.87 | 5.22 | 5.35 |
| $CD3^-CD16^+CD56^+$ | 295.88 | 6.03 | 6.21 |

## Precision (repeatability), control material (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A 21-day single-site precision study was performed to assess repeatability and within-site precision when samples were prepared and acquired on the BD FACSLyric™ flow cytometer with BD FACSDuet™ sample preparation system using control material. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three BD FACSDuet™ systems, each integrated with a BD FACSLyric™ flow cytometer, and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), stained in duplicate using

three lots of BD Multitest™ CD3/CD16+CD56/CD45/CD19. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts, respectively.

**Table 16** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 77.47 | 0.86 | 0.86 |
| $CD19^+$ | 11.93 | 0.64 | 0.64 |
| $CD3^-CD16^+CD56^+$ | 9.98 | 0.55 | 0.55 |

**Table 17** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 63.66 | 1.00 | 1.07 |
| $CD19^+$ | 18.24 | 0.71 | 0.72 |
| $CD3^-CD16^+CD56^+$ | 16.87 | 0.72 | 0.74 |

**Table 18** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,750.80 | 4.59 | 6.84 |
| $CD19^+$ | 269.47 | 6.93 | 8.53 |
| $CD3^-CD16^+CD56^+$ | 225.59 | 6.95 | 8.45 |

**Table 19** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 735.99 | 4.05 | 4.94 |
| $CD19^+$ | 210.89 | 5.69 | 6.37 |
| $CD3^-CD16^+CD56^+$ | 195.06 | 5.40 | 6.21 |

## Precision (repeatability), peripheral blood (standalone BD FACSLyric™ flow cytometer)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 53 donor samples. Each donor sample was stained in duplicate using the BD Multitest™

CD3/CD16+CD56/CD45/CD19 reagent in BD Trucount™ Tubes and run on 12 instruments for a total of 24 runs per sample.

**Table 20** Repeatability and within-site precision of lymphocyte subset percentages

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 73.64 | 0.97 | 0.97 |
| $CD19^+$ | 13.02 | 0.67 | 0.67 |
| $CD3^-CD16^+CD56^+$ | 12.37 | 0.71 | 0.71 |

**Table 21** Repeatability and within-site precision of lymphocyte subset absolute counts

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,396.78 | 4.17 | 4.26 |
| $CD19^+$ | 229.21 | 7.32 | 7.47 |
| $CD3^-CD16^+CD56^+$ | 215.01 | 7.84 | 7.94 |

## Precision (repeatability), peripheral blood (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 27 donor specimens. Each donor specimen was stained in duplicate using three lots of BD Multitest™ CD3/CD16+CD56/CD45/CD19 in BD Trucount™ Tubes and run on three BD FACSDuet™ instruments, each integrated with a BD FACSLyric™ flow cytometer, for a total of 18 runs per sample.

**Table 22** Repeatability and within-site precision of lymphocyte subset percentages

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| $CD3^+$ | 73.73 | 0.97 | 0.98 |
| $CD19^+$ | 11.97 | 0.67 | 0.67 |
| $CD3^-CD16^+CD56^+$ | 10.66 | 0.81 | 0.83 |

**Table 23** Repeatability and within-site precision of lymphocyte subset absolute counts

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| $CD3^+$ | 1,584.20 | 4.50 | 4.84 |
| $CD19^+$ | 246.37 | 7.55 | 7.80 |
| $CD3^-CD16^+CD56^+$ | 217.20 | 10.46 | 11.02 |

## Precision (reproducibility), control material (standalone BD FACSLyric™ flow cytometer)

A study was performed at four sites to assess reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19. A single lot of each control material, CD-Chex Plus® control (CDN) and CD-Chex Plus® CD4 Low control (CDL), was provided to each of the sites. For each type of control material, three replicates were stained using BD Multitest™ CD3/CD16+CD56/CD45/CD19. Two separate runs were analyzed during each of 5 nonconsecutive testing days.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for reproducibility of lymphocyte subset percentages and absolute counts, respectively.

**Table 24** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 76.84 | 1.05 |
| $CD19^+$ | 12.02 | 0.66 |
| $CD3^-CD16^+CD56^+$ | 10.30 | 0.62 |

**Table 25** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 57.10 | 1.30 |
| $CD19^+$ | 21.74 | 0.83 |
| $CD3^-CD16^+CD56^+$ | 19.44 | 1.06 |

**Table 26** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 1,748.31 | 4.80 |
| $CD19^+$ | 273.58 | 7.23 |
| $CD3^-CD16^+CD56^+$ | 234.28 | 7.50 |

**Table 27** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 884.57 | 4.82 |
| $CD19^+$ | 336.79 | 5.71 |
| $CD3^-CD16^+CD56^+$ | 301.25 | 7.40 |

## Precision (reproducibility), control material (BD FACSLyric™ flow cytometer) with BD FACSDuet™ system)

A study was performed at three sites to assess reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19. A single lot of each process control, CD-Chex Plus CD4 Low control and CD-Chex Plus control, was provided to each site. The control samples were stained using three lots of BD Multitest™ CD3/CD16+CD56/CD45/CD19 with one lot of BD Trucount™ Tubes using the BD FACSDuet™ sample preparation system and automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using the BD FACS™ Universal Loader. Two separate runs were performed each day. Results obtained over 15 non-consecutive test days were analyzed.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for reproducibility (total precision) of lymphocyte subset percentages and absolute counts, respectively.

**Table 28** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset percentages in normal analyte concentration (CDN) )

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 75.94 | 0.84 |
| $CD19^+$ | 12.19 | 0.57 |
| $CD3^-CD16^+CD56^+$ | 11.17 | 0.58 |

**Table 29** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | SD |
|---|---|---|
| $CD3^+$ | 57.08 | 1.06 |
| $CD19^+$ | 21.46 | 0.84 |
| $CD3^-CD16^+CD56^+$ | 20.16 | 0.85 |

**Table 30** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 1,973.79 | 6.11 |
| $CD19^+$ | 317.00 | 8.36 |
| $CD3^-CD16^+CD56^+$ | 290.39 | 8.15 |

**Table 31** Reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 for lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | %CV |
|---|---|---|
| $CD3^+$ | 958.41 | 6.21 |
| $CD19^+$ | 360.46 | 7.21 |
| $CD3^-CD16^+CD56^+$ | 338.44 | 7.53 |

## Linearity (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

Linearity was assessed for the BD FACSLyric™ flow cytometer, with and without an integrated BD FACSDuet™ system, using triplicate measurements of 11 equally spaced concentrations of WBCs. Lymphocyte subsets were observed to be linear across the following ranges.

**Table 32** Linear ranges of lymphocyte subsets (BD FACSLyric™ flow cytometer with and without BD FACSDuet™)

| Lymphocyte subset | Range (cells/µL) | |
|---|---|---|
| | Standalone BD FACSLyric™ | BD FACSLyric™ with BD FACSDuet™ |
| $CD3^+$ | 8–5,215 | 5–5,194 |
| $CD19^+$ | 0–2,601 | 0–2,237 |
| $CD3^-CD16^+CD56^+$ | 2–1,396 | 1–1,419 |

## Measuring range (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

The analytical measurement range (AMR) for BD Multitest™ CD3/CD16+CD56/CD45/CD19 on the BD FACSLyric™ flow cytometer was determined. To establish the measuring range of the BD Multitest™ CD3/CD16+CD56/CD45/CD19, data was taken from the following:

- The LOQ studies using the BD FACSLyric™ flow cytometer with and without the BD FACSDuet™ system.
- The method comparison study between the BD FACSLyric™ and the BD FACSCanto™ II flow cytometers.
- The method comparison study between the standalone BD FACSLyric™ flow cytometer and the BD FACSLyric™ with BD FACSDuet™ system.

The lower end of the AMR was determined based on results from the limit of quantitation (LoQ) studies and the upper end of the AMR was determined based on results from the method comparison studies.

**Table 33** AMR of lymphocyte subsets (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

| Lymphocyte subset | Analytical measuring range (cells/µL) |
|---|---|
| $CD3^+$ | 19–5,000 |
| $CD19^+$ | 15–2,000 |
| $CD3^-CD16^+CD56^+$ | 13–1,200 |

## BD FACSCanto™ II Flow Cytometer

### Method comparison, BD FACSCanto™ II vs BD FACSCanto™ flow cytometer

Lymphocyte subset percentages and absolute counts were enumerated with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in BD Trucount™ Tubes and analyzed on a BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software version 2.1. The results were compared with results from the same samples analyzed on the BD FACSCanto™ flow cytometer using BD FACSCanto™ Clinical Software version 2.0. Peripheral blood samples were collected at random at one clinical laboratory. Regression statistics are reported in the following table.

**Table 34** Regression analysis for subset absolute counts and percentages

| Lymphocyte subset | N | Units | $R^2$ | Slope | Intercept | Range |
|---|---|---|---|---|---|---|
| Average $CD3^+$ | 104 | % | 0.984 | 0.97 | 2.72 | 51–92 |
| | | cells/µL | 0.991 | 0.97 | 27.59 | 221–3,872 |
| $CD19^+$ | 104 | % | 0.986 | 0.97 | 0.32 | 0–39 |
| | | cells/µL | 0.979 | 0.97 | 2.37 | 0–834 |
| $CD3^-CD16^+CD56^+$ | 104 | % | 0.957 | 0.93 | 0.19 | 1–32 |
| | | cells/µL | 0.961 | 0.88 | 10.56 | 20–606 |

### Precision (repeatability), control material (BD FACSCanto™ II flow cytometer)

A 21-day single-site study was conducted assess repeatability precision. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three instruments and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low (CDL) control, stained in duplicate using one lot of BD Multitest™ CD3/CD16+CD56/CD45/CD19. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables present SDs and CVs for within-device precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

**Table 35** Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| Average $CD3^+$ | 73.0 | 0.63 | 0.67 |
| $CD19^+$ | 15.4 | 0.54 | 0.56 |
| $CD3^-CD16^+CD56^+$ | 10.6 | 0.51 | 0.52 |

**Table 36** Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

| Lymphocyte subset | Mean (%) | Repeatability (SD) | Within-site precision (SD) |
|---|---|---|---|
| Average $CD3^+$ | 54.1 | 0.96 | 0.98 |
| $CD19^+$ | 26.1 | 0.86 | 0.86 |
| $CD3^-CD16^+CD56^+$ | 18.2 | 0.87 | 0.87 |

**Table 37** Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| Average $CD3^+$ | 2,105.4 | 2.7 | 2.9 |
| $CD19^+$ | 443.5 | 5.5 | 5.6 |
| $CD3^-CD16^+CD56^+$ | 306.3 | 6.0 | 6.0 |

**Table 38** Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

| Lymphocyte subset | Mean (cells/µL) | Repeatability (%CV) | Within-site precision (%CV) |
|---|---|---|---|
| Average $CD3^+$ | 1,086.0 | 3.5 | 3.6 |
| $CD19^+$ | 526.1 | 6.2 | 6.4 |
| $CD3^-CD16^+CD56^+$ | 367.1 | 5.9 | 6.1 |

## Linearity (BD FACSCanto™ II flow cytometer)

Linearity of the BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagent was assessed for the BD FACSCanto™ II system within a WBC range of 0 to $3.8 \times 10^4$ cells/µL. Results were observed to be linear across the following ranges.

| Lymphocyte subset | Range (cells/µL) |
|---|---|
| Average $CD3^+$ | 4–5998 |
| $CD19^+$ | 0–857 |
| $CD3^-CD16^+CD56^+$ | 0–447 |

## 11. TROUBLESHOOTING

| Problem | Possible Cause | Solution |
|---|---|---|
| Poor resolution between debris and lymphocytes. | Cell interaction with other cells and platelets. | Prepare and stain another sample. |
| | Rough handling during cell preparation. | Check cell viability. Centrifuge cells at lower speed. |
| | Inappropriate instrument settings. | Follow proper instrument setup procedures. Optimize instrument settings as required. |
| Staining dim or fading. | Cell concentration too high at staining step. | Check and adjust cell concentration or sample volume. Stain with fresh sample. |
| | Insufficient reagent. | Repeat staining with increased amount of antibody. |
| | Cells not analyzed within 24 hours of staining. | Repeat staining with fresh sample. Analyze promptly. |
| Few or no cells. | Cell concentration too low. | Resuspend fresh sample at a higher concentration. Repeat staining and analysis. |
| | Cytometer malfunctioning. | Troubleshoot instrument. |

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## NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

## 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.



## HISTORY

| Revision | Date | Changes made |
|---|---|---|
| 23-5345(10) | 2022-12 | Updated to meet requirements of Regulation (EU) 2017/746. |
| 23-5345(11) | 2023-07 | Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary. |

**Symbols Glossary**

Please refer to product labeling for applicable symbols.

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|  | Authorized representative in the European Community |
|  | Authorised representative in Switzerland |
|  | Date of manufacture |
|  | Use-by date |
|  | Batch code |
|  | Catalogue number |
|  | Serial number |
|  | Sterile |
|  | Sterilized using aseptic processing techniques |
|  | Sterilized using ethylene oxide |
|  | Sterilized using irradiation |
|  | Sterilized using steam or dry heat |
|  | Do not resterilize |
|  | Non-sterile |
|  | Do not use if package is damaged and consult *instructions for use* |
|  | Sterile fluid path |
|  | Sterile fluid path (ethylene oxide) |
|  | 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 |
|  | Contains or presence of natural rubber latex |
|  | In vitro diagnostic medical device |
|  | Negative 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 |
|  | Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP) |
|  | Collect separately<br>Indicates separate collection for waste of electrical and electronic equipment required. |
|  | CE marking; Signifies European technical conformity |
|  | Device for near-patient testing |
|  | Device for self-testing |
|  | This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner." |
|  | Country of manufacture<br>"CC" shall be replaced by either the two letter or the three letter country code. |
|  | Collection time |
|  | Cut |
|  | Peel here |
|  | Collection date |
|  | Keep away from light |
|  | Hydrogen gas is generated |
|  | Perforation |
|  | Start panel sequence number |
|  | End panel sequence number |
|  | Internal sequence number |
|  | <Box #> / <Total Boxes> |
|  | Medical device |
|  | Contains hazardous substances |
|  | Ukrainian conformity mark |
|  | Meets FCC requirements per 21 CFR Part 15 |
|  | UL product certification for US and Canada |
|  | Unique device identifier |
|  | Importer |
|  | Place patient label in framed area only |
|  | Magnetic resonance (MR) safe |
|  | Magnetic resonance (MR) conditional |
|  | Magnetic resonance (MR) unsafe |
|  | For use with |
|  | This Product Contains Dry Natural Rubber |
|  | For Export Only |
|  | Instruments |

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

L006715(08) 2023-03

## CONTACT INFORMATION


**Becton, Dickinson and Company**
**BD Biosciences**
155 North McCarthy Boulevard
Milpitas, California 95035 USA

EC REP **Becton Dickinson Ireland Ltd.**
Donore Road, Drogheda
Co. Louth, A92 YW26
Ireland

**Becton Dickinson Distribution Center NV**
Laagstraat 57
9140 Temse, Belgium

CH REP **BD Switzerland Sàrl**
Route de Crassier 17
Business Park Terre-Bonne
Bâtiment A4
1262 Eysins
Switzerland

**Becton Dickinson AG**
Binningerstrasse 94
4123 Allschwil
Switzerland

**BD Biosciences**
**European Customer Support**
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand Distributors:

**Becton Dickinson Pty Ltd.**
66 Waterloo Road
Macquarie Park NSW 2113
Australia

**Becton Dickinson Limited**
14B George Bourke Drive
Mt. Wellington Auckland 1060
New Zealand


Technical Service and Support: In the United States contact BD at 1.877.232.8995 or bdbiosciences.com.
For regions outside the United States, contact your local BD representative or bdbiosciences.com.

ClinicalApplications@bd.com

eifu.bd.com

# BD™ CS&T Beads

| Catalog No. | Tests |
|---|---|
| 656504 | 50 |
| 656505 | 150 |

4/2016 23-14666-01

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**Becton, Dickinson and Company**
**BD Biosciences**
2350 Qume Drive
San Jose, CA 95131 USA

EC REP

**Benex Limited**
Pottery Road, Dun Laoghaire,
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

**BD Biosciences**
**European Customer Support**
Tel +32.2.400.98.95
Fax +32.2.401.70.94
help.biosciences@europe.bd.com

**Becton Dickinson Pty Ltd,**
4 Research Park Drive,
Macquarie University Research Park,
North Ryde NSW 2113, Australia

**Becton Dickinson Limited,**
8 Pacific Rise, Mt. Wellington,
Auckland, New Zealand

bdbiosciences.com
ClinicalApplications@bd.com

## 1. INTENDED USE

BD™ CS&T beads are used on a BD flow cytometer to provide a standardized method to perform quality control of the instrument's optics, electronics, and fluidics, and for adjusting fluorescence compensation. On some BD instruments, BD CS&T beads are also used for adjusting detector voltages.

## 2. SUMMARY AND EXPLANATION

BD CS&T beads are a suspension of fluorospheres with uniform and stable size and fluorescence intensity. The beads are used for instrument quality control (QC) to characterize, track, and report performance measurements of supported flow cytometers. The cytometer's software displays current bead data in plots. Forward scatter (FSC) and side scatter (SSC) identify bead populations based on relative size.

The beads enable the software to measure detector performance and are used to measure the sensitivity of each fluorescence detector. Sensitivity is a measure of the cytometer's ability to resolve dimly stained cells. In addition, the beads are used to optimize the compensation settings each time instrument QC is run.

The software calculates the bright bead median, bright beads %rCV (robust coefficient of variation), and instrument sensitivity for FSC, SSC, and each fluorescence parameter, and compares them to expected values for the bead lot. The rCV measures cytometer alignment.

Daily measurements are automatically entered into Levey-Jennings plots. This allows you to monitor instrument

performance measurements over time and detect potential problems.

For the BD FACSLyric™ flow cytometer, BD CS&T beads are also used for adjusting detector voltages.

## 3. PRINCIPLES OF THE PROCEDURE

BD CS&T beads consist of equal quantities of 3-µm bright, 3-µm mid, and 2-µm dim polystyrene beads. The beads are dyed with fluorochromes. Fluorescence intensity is measured by the cytometer's detectors, processed by the electronics, and displayed and analyzed by the software.

The cytometer's software reads the bead lot file and calculates the target marker position or target fluorescence intensity for the beads. It then calculates the median and %rCV for bright beads.

## 4. REAGENTS

### Reagents provided

BD CS&T beads are supplied in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide as follows.

- Two 3-mL vials (50 tests)
- Six 3-mL vials (150 tests)

Each 3-mL vial contains sufficient beads to run 25 tests.

### Reagents or materials required but not provided

- Disposable 12 × 75-mm capped polystyrene test tubes
- Filtered deionized (DI) water, to dilute the beads (BD FACSVia™ system only)
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent, to dilute the beads (BD FACSLyric flow cytometer only)

### Precautions

- Avoid exposing BD CS&T beads to direct light.
- Do not run BD CS&T beads without first diluting them with the proper diluent, as directed in the Procedure section.
- Do not use BD CS&T beads beyond their expiration date or beyond the day-of-use stability period after dilution. Beads used beyond their stability period begin to lose fluorescence, which can result in failed instrument QC.
- BD CS&T beads contain sodium azide as a preservative.

### Storage and handling

- Store vials at 2°C–8°C and protect from light. Do not use after the expiration date shown on the label.

## 5. INSTRUMENTS

BD CS&T beads are for use on the following:

- BD FACSVia flow cytometer
- BD FACSLyric flow cytometer

## 6. PROCEDURE

### Adding or importing bead lot information

Add bead lot ID information by scanning the bead lot file card in this kit.

If you do not have a barcode scanner, import bead lot ID information from the BD Biosciences website.

1. Visit bdbiosciences.com and select **Support** from the menu bar.

   The **Services** web page opens.

2. From **Top Support Links** in the right panel, select **Bead Lot Files:** for the appropriate software.
3. Follow the installation instructions on the website to download and import the appropriate bead lot file into the software.

### Preparing a BD CS&T bead suspension

Carefully read the Precautions and Storage and handling statements in the Reagents section.

To prepare the BD CS&T beads for acquisition:

1. Label a 12 × 75-mm capped polystyrene tube.
2. Thoroughly mix the BD CS&T beads vial. Invert the vial 10 times, or vortex the vial at medium speed for 5–10 seconds.
3. Prepare diluted beads according to Table 1 for the system and application you are running.

   **NOTE** Avoid dripping the beads down the side of the tube when diluting them. This can lead to low bead counts during acquisition.

   **NOTE** Do not dilute BD CS&T beads more than recommended.
4. Vortex the tubes gently before use.

After dilution, the beads are stable for:

- 8 hours at 2°C–25°C on the BD FACSVia system
- 8 hours at 15°C–25°C, or 24 hours at 2°C–8°C on the BD FACSLyric flow cytometer.

**NOTE** Keep the diluted bead suspension protected from light. Some of the dyes used to manufacture the beads are light sensitive. Fluorescence levels can change if beads are exposed to direct light for longer than 20 minutes.

**Table 1** BD CS&T beads preparation

| For... | | Add... | | | | |
|---|---|---|---|---|---|---|
| **Task** | **Cytometer** | **Diluent** | **Diluent volume (µL)** | **Beads (No. of drops)** | **To tube labeled** | **How often** |
| Instrument QC (IQC) | BD FACSVia | Filtered DI water | 500 | 2 | IQC | • Daily<br>• When recommended by BD<br>• To transfer bead lots |

**Table 1** BD CS&T beads preparation

| For... | | Add... | | | | |
|---|---|---|---|---|---|---|
| **Task** | **Cytometer** | **Diluent** | **Diluent volume (µL)** | **Beads (No. of drops)** | **To tube labeled** | **How often** |
| Performance QC[a] (PQC) | BD FACSLyric | BD FACSFlow sheath fluid | 500 | 2 | PQC | Daily |
| Update reference settings | | | 500 | 2 | Ref | Every 60 days |
| Bead lot transfer | | | 500 | 2 | Old lot | Before using a new lot |
| | | | 500 | 2 | New lot | |
| Characterization QC (CQC) | | | 1,000 | 4 | CQC | • Every 6 months<br>• After service or maintenance<br>• When recommended by BD |
| Laser setup | | | 1,000 | 4 | Laser | As necessary |

a. Assay and tube settings are automatically updated when running performance QC.

### Performing QC on the instrument using BD CS&T beads

Run instrument QC according to Table 1. See the instrument's Instructions For Use (IFU) for instructions on installing a bead lot file and performing any of the tasks outlined in Table 1.

## 7. RESULTS

### Reviewing the Instrument QC Report

The Instrument QC Report contains the cytometer serial number, software version, BD CS&T bead lot information, bright bead median, bright beads %rCV, instrument sensitivity, and a pass or fail result for each parameter. A passing result for every parameter is required for instrument QC to pass. A failure for any parameter results in failure of instrument QC. For troubleshooting any QC messages, see the cytometer's IFU.

When using the BD FACSVia system, we recommend that you visually review the marker positions for scatter and bright bead peaks and adjust the markers to surround the bead population, as necessary.

## 8. LIMITATIONS

- BD CS&T beads are intended for use with supported flow cytometers and their applicable software.
- BD CS&T beads should not be used to support quantitative fluorescence measurements in a flow cytometer.
- BD CS&T beads are for instrument QC and setup only.

## 9. PERFORMANCE CHARACTERISTICS

Performance of the BD CS&T beads was established by testing at BD Biosciences laboratories in San Jose, CA, USA.

### Accuracy

Assay settings and tube settings were determined three times on one BD FACSLyric flow cytometer using one lot of BD CS&T beads. For each

fluorescence channel, the bright bead median fluorescence intensity (MFI) value (Actual), generated from the assay setup reports, was compared with the lot-specific bright bead MFI value (Target).

Accuracy was calculated as the percent difference between the bright bead MFI values of the Actual and the bright bead MFI values of the Target. See Table 2.

**Table 2** Accuracy of cytometer setup using BD CS&T beads[a]

| Parameter | Bright bead MFI | | % Difference |
|---|---|---|---|
| | Target | Actual | |
| FSC | 17,991 | 17,992 | 0.006 |
| SSC | 126,269 | 126,459 | 0.150 |
| FITC | 5,952 | 5,930 | –0.370 |
| PE | 12,719 | 12,700 | –0.149 |
| PerCP-Cy5.5 | 17,875 | 17,950 | 0.420 |
| PE-Cy7 | 16,237 | 16,250 | 0.080 |
| APC | 40,693 | 40,901 | 0.511 |
| APC-R700[b] | 42,873 | 42,951 | 0.182 |
| APC-Cy7 | 85,174 | 85,397 | 0.262 |
| V450[a] | 6,203 | 6,219 | 0.258 |
| V500-C[a] | 24,488 | 24,483 | –0.020 |
| BV605[a] | 6,423 | 6,393 | –0.467 |

a. The data presented are from one run with LNW tube settings. Results from LW tube settings and the other runs were similar.
b. BD Horizon™ APC-R700, BD Horizon™ V450, BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 605

## Reproducibility

Instrument CQC was run on two BD FACSLyric flow cytometers. Two operators performed two runs of instrument PQC in duplicate on each instrument every day for a period of eight days using one lot of BD CS&T beads. Percent coefficient of variation (%CV) of the median MFI values for each channel in high sensitivity and normal modes was used to verify reproducibility. See Table 3.

**Table 3** Reproducibility of BD CS&T beads (Operator/instrument-to-operator/instrument, day-to-day, tube-to-tube)[a]

| Parameter | %CV (High sensitivity) | %CV (Normal) |
|---|---|---|
| FSC | 0.98 | 0.94 |
| SSC | 0.73 | 0.61 |
| FITC | 0.46 | 0.22 |
| PE | 0.41 | 0.24 |
| PerCP-Cy5.5 | 0.88 | 0.78 |
| PE-Cy7 | 1.28 | 1.23 |
| APC | 0.77 | 0.80 |
| APC-R700 | 0.60 | 0.61 |
| APC-Cy7 | 0.67 | 0.71 |
| V450 | 0.67 | 0.65 |
| V500-C | 0.53 | 0.52 |
| BV605 | 0.54 | 0.48 |

a. The data presented are from one instrument. Results from the other instrument were similar.

## Repeatability

Instrument CQC and PQC were each performed ten times on two BD FACSLyric flow cytometers using two lots of BD CS&T beads to assess run-to-run repeatability. The %CV of the bright beads %rCV (resolution), Br (background), minimum linearity, maximum linearity, and SDen (standard deviation of the electronic noise) were used to verify run-to-run repeatability. See Table 4.

**Table 4** Run-to-run repeatability of instrument CQC using BD CS&T beads[a]

| Parameter | Bright bead rCV | | SD for Br <100 | %CV for Br ≥100 | SD for linearity minimum (<500) | %CV for linearity maximum | %CV for SDen |
|---|---|---|---|---|---|---|---|
| | %CV of rCV | SD of rCV <2% | | | | | |
| FSC | NA | 0.07 | NA | NA | NA | NA | NA |
| SSC | NA | 0.03 | NA | NA | NA | NA | NA |
| FITC | NA | 0.05 | NA | 5.01 | 24.04 | 0.14 | 2.87 |
| PE | NA | 0.06 | NA | 4.97 | 23.84 | 0.15 | 3.09 |
| PerCP-Cy5.5 | 2.36 | NA | 2.67 | NA | 16.05 | 0.15 | 2.43 |
| PE-Cy7 | 0.88 | NA | 0 | NA | 11.55 | 0.20 | 1.86 |
| APC | NA | 0.07 | 1.42 | NA | 22.41 | 0.32 | 1.64 |
| APC-R700 | NA | 0.06 | 4.10 | NA | 29.58 | 0.26 | 2.14 |
| APC-Cy7 | NA | 0.09 | 26.64 | NA | 22.23 | 0.34 | 1.63 |
| V450 | 0.96 | NA | NA | 7.0 | 23.20 | 0.25 | 2.82 |
| V500-C | 1.58 | NA | NA | 6.34 | 46.83 | 0.32 | 2.25 |
| BV605 | 1.44 | NA | 2.67 | NA | 47.54 | 0.26 | 1.45 |

a. The data presented are for one lot of BD CS&T beads run on one instrument. Results for the remaining lots of beads and instruments were similar.

## TROUBLESHOOTING

| Problem | Possible Causes | Solution |
|---|---|---|
| No beads detected | Beads not mixed prior to diluting, beads are too dilute, there is debris in the bead suspension, incorrect beads were used, beads diluted in wrong diluent, beads exposed to light | 1. Vortex the bead vial.<br>2. Prepare a fresh suspension of beads.<br>3. Re-run instrument QC. |
| | Air bubbles in the flow cell or sheath filter | 1. For:<br>• BD FACSVia, perform a backflush or SIP clean.<br>• BD FACSLyric, perform a SIT flush.<br>2. Vortex the tube.<br>3. Re-run the tube. |
| | Sheath filter is not filled with fluid | • For BD FACSVia, perform the two-month maintenance procedure.<br>• For BD FACSLyric, purge the sheath filter. |
| No beads detected | Clogs within the sample path and fluidic lines | 1. For:<br>• BD FACSVia, perform a backflush or SIP clean.<br>• BD FACSLyric, perform a SIT flush.<br>2. Vortex the tube.<br>3. Re-run the tube. |
| | Optics are out of alignment | • Contact BD Biosciences. |
| Performance check completed with QC messages | Bead gates and markers are not properly adjusted to encapsulate results | For BD FACSVia, review instrument QC results and adjust the CS&T Bead gates for scatter and fluorescence. |
| | Values for any measurements used to check cytometer performance are not within parameters required for instrument QC to pass (see Reagents section) | Prepare a fresh suspension of beads and re-run instrument QC. |
| | | 1. For:<br>• BD FACSVia, perform the two-month maintenance procedure.<br>• BD FACSLyric, perform the monthly cleaning procedure.<br>2. Re-run the tube. |
| | | Review the instrument QC report to determine whether the specific warnings impact the assay, then continue. |
| | | Contact BD Biosciences. |
| Performance check failure | Value(s) for any of the measurements used to check the cytometer performance are not within parameters required for instrument QC to pass (see Section 7) | 1. Prepare a fresh suspension of beads.<br>2. Re-run the performance check. |
| | | Perform the monthly cleaning procedure. |
| | Improper ratio of 2 µm/3 µm beads due to inadequate mixing of beads | 1. Prepare a fresh suspension of beads.<br>2. Re-run the performance check.<br>3. If QC fails again, prepare beads from a new vial and be sure to thoroughly vortex the vial prior to use. See Preparing a BD CS&T bead suspension. |

For additional troubleshooting assistance, see the cytometer's IFU or contact your local BD Biosciences representative.

## 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.

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