

BD DNA QC Particles

Catalog No. 349523

25 Tests

Research Applications

Research applications include:

- Setup and verification of the doublet discrimination function of BD FACSCalibur™ and other BD flow cytometers equipped with a doublet discrimination module (DDM) for DNA analysis, and for monitoring daily instrument performance.
- Verification of instrument performance and quality control of other flow cytometers; all performance specifications will need to be determined for each individual case, although the particles will meet the stability criteria as stated.

Summary and Explanation

Flow cytometric analysis of cellular DNA content has become an increasingly important research tool for measuring and identifying abnormal cell populations. Examination of cellular DNA content can provide information for use in cell-cycle analysis, as well as for establishing a DNA index¹ for diploid and aneuploid cells in cancer studies.²⁻⁶ As a result, uniform biological particles have become a necessary component for setup, control, and troubleshooting of flow cytometers used for DNA analysis.

BD® DNA QC Particles provide a means for testing and documenting optimal performance on BD or other flow cytometers. The kit is composed of four reagents. Vial A contains chicken erythrocyte nuclei (CEN), used in setting instrument photomultiplier tube (PMT) voltages and amplifier gains, and providing information regarding instrument linearity and resolution. Vial B contains calf thymocyte nuclei (CTN), a cycling cell component that allows assessment of proper function of the doublet discrimination module (DDM) or pulse processing. Vial C contains 2-µm fluorescent beads, which function as a stable particle to verify instrument alignment. Vial D contains propidium iodide (PI), a DNA stain.

Description

CEN are prepared by treating chicken blood with detergent, followed by fixation of the isolated nuclei in ethanol. These fixed nuclei may be used as instrument performance particles to set up flow cytometers for DNA analysis.

CEN preparation is designed to contain single nuclei, doublets, triplets, and some larger aggregates. The CEN are intended for checking instrument linearity and resolution. When stained with propidium iodide, these aggregates result in four or more reference peaks in the fluorescence 2-area or fluorescence 2-height. If the instrument response is linear and the nuclei have been correctly stained, the channel values of the means should be approximately 2, 3, and 4 times greater than the singlet peak.

CTN are prepared by treating calf thymocytes with detergent, followed by fixation of the isolated nuclei in formaldehyde. CTN provide a stable source of nuclei with all phases of the cell cycle present. Most of the nuclei are in the G₀/G₁ stage, and a smaller portion exists in the S and G₂+M stages. Doublets of the G₀/G₁ nuclei also exist and can be discriminated from true G₂+M singlets with the use of a DDM or pulse processing. The DDM enables doublets of cells in G₀/G₁ phase, and other aggregates, to be distinguished from single cells in G₂+M phase, thus allowing improved cell-cycle estimates of the percentage of cells in G₂+M phase (4 N DNA content).

NOTE The degree of staining of CTN provided will be less than would be obtained with fresh nuclei, since formaldehyde fixation reduces CTN's ability to stain with PI.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

The PI stain intercalates into the DNA of both the CEN and CTN. Blue light (488 nm) excites this fluorochrome, which fluoresces red with a maximum emission at approximately 623 nm. The intensity of the fluorescence is a function of the DNA content of the cell. (See Limitations).

The 2- μ m fluorescent beads may be used as a check of cytometer alignment. They fluoresce in both FL1 and FL2, thereby providing a means of verifying instrument performance independent of stain or sample preparation. If the CV of the CEN or the CTN does not meet specification, we recommend running the 2- μ m beads.

Reagents

Reagents Provided, Sufficient for 25 Tests

- Vial A: 1-mL suspension of CEN in buffer and ethanol
- Vial B: 1-mL suspension of CTN in buffer with formaldehyde and 0.01% thimerosal
- Vial C: 1-mL suspension of 2- μ m fluorescent beads in buffer with gelatin and 0.1% sodium azide
- Vial D: 50 mL of 50- μ g/mL solution of PI in buffer (25 tests using CEN and CTN)

Reagents and Materials Required But Not Provided

- 12 x 75-mm disposable test tubes
- Precision micropipettor, 1-mL capacity
- Precision micropipettor, 50- μ L capacity
- Vortex mixer
- Ice bath with cover
- 1X phosphate-buffered saline (PBS), calcium and magnesium free. Filter through a 0.2- μ m filter before use. Store at 2–8 °C.
- 6-mL 12 x 75-mm Falcon® tube with 35- μ m strainer cap

Handling and Storage

Store vials at 2–8 °C. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed. Keep the reagent vials dry.

Precautions

- There is a tendency for the CEN (vial A) to settle during storage. This can be corrected by gently vortexing before use. Vortexing too vigorously will separate desired aggregates. It is critical that CEN be stored at 2–8 °C, since these particles are sensitive to thermal degradation.
- CTN (vial B) tend to aggregate during storage and should be vigorously vortexed to separate these aggregates.
- The 2- μ m beads (vial C) must be protected from prolonged exposure to light.
- Do not expose stained CEN or CTN to prolonged light during storage or incubation.
- Incubation times or temperatures other than those specified may lead to erroneous results.
- Propidium iodide solution (vial D) must be protected from prolonged exposure to light. Deterioration in stain performance has been observed with short periods of room temperature storage. Therefore, stained samples must be stored at 2–8 °C in the dark until analysis (maximum 12 hours).

Warnings

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, eye wear, and gloves.

The reagent contains 43.982% Ethanol, CAS number 64-17-5, EC Number 200-578-6; 0.187% Formaldehyde, CAS number 50-00-0, EC Number 200-001-8; 0.0095% Thimerosal, CAS Number 54-64-8, EC Number 200-210-4; 4.630% 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol, CAS Number 9002-93-1, EC Number

255-695-5. The reagent is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Danger H226: Flammable liquid and vapor. H317: May cause an allergic skin reaction. H350: May cause cancer. H318: Causes serious eye damage. H400: Very toxic to aquatic life. H411: Toxic to aquatic life with long lasting effects.
Prevention	P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P240: Ground and bond container and receiving equipment. P241: Use explosion-proof [electrical/ventilating/lighting/...] equipment. P242: Use non-sparking tools. P243: Take action to prevent static discharges. P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P370+P378: In case of fire: Use water spray, fog, CO2, dry chemical, or alcohol resistant foam. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P363: Wash contaminated clothing before reuse. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see supplemental first aid instructions on this label). P308+P313: IF exposed or concerned: Get medical advice/attention. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER/doctor. P391: Collect spillage.
Storage	P403: Store in a well-ventilated place. P405: Store locked up

	Danger
	P235: Keep cool.
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.

Go to regdocs.bd.com to download the Safety Data Sheet.

Instrument

BD DNA QC Particles are designed for use on BD FACSCalibur™ and other flow cytometers with appropriate computer hardware, software, and gating electronics. The flow cytometer employed must be equipped with standard fluorescence and forward scatter detection. A DDM or other electronic pulse processor capability is required for doublet discrimination.

BD CellQuest™ software and the DNA Experiment Document on the Macintosh® platform may be used to acquire DNA samples for analysis using an appropriate DNA analysis software package. Refer to the cytometer user's guide for software to use with other instruments.

Reagent preparation

CEN

Prior to staining, gently vortex the nuclei. Pipet 40 µL of CEN from vial A into a tube containing 1 mL of PI stain solution from vial D, cap, and gently vortex. Incubate the sample for 10 minutes at room temperature, protected from light. Keep the sample in the dark at 2–8 °C or in a covered ice bath until ready for analysis. PI-stained CEN are typically stable up to 4 hours when stored in the dark at 2–8 °C.

CTN

Vigorously vortex the bottle of nuclei. Pipet 40 µL of CTN from vial B into a tube containing 1 mL of PI stain solution from vial D, cap, and gently vortex. Incubate the sample for 10 minutes at room temperature, protected from light. Keep the sample in the dark at 2–8 °C or in a covered ice bath until ready for analysis. PI-stained CTN are typically stable up to 4 hours when stored in the dark at 2–8 °C.

2-µm Beads

Vigorously vortex the bottle of beads. Dispense one drop of beads from vial C into a tube containing 1 mL of filtered PBS, cap, and gently vortex to disperse. Diluted beads are stable for 12 hours when stored in the dark at 2–8 °C or in a covered ice bath, until ready for analysis.

Procedure for Setup on the BD FACSCalibur™

Before setting up your flow cytometer, turn on the flow cytometer, Macintosh computer system, and printer. For specific information, refer to the BD CellQuest™ DNA Experiment Document User's Guide.

1. Insert the DNA Application disk into the floppy disk drive.
2. Double-click the DNA Application disk icon to view the disk contents in a window. Copy the disk contents onto the hard drive.
3. Double-click the DNA QC folder.
4. Double-click the DNA Experiment Document file.

Installing the flow cytometer instrument settings

NOTE As you open the various windows, you can reposition the windows on the desktop. You can print the pages of your Experiment Document by selecting Print from the File menu.

1. Choose **Instrument Settings** from the **Cytometer** menu.
2. Click **Open**.
3. Select the appropriate Instrument Settings file and click **Open**.
4. Click **Set**.
5. Click **Done**.
6. Choose **Detectors/Amps** from the **Cytometer** menu.

Acquisition setup

Before beginning acquisition, set up your folder, folder location, and file name as follows.

1. Choose **Parameter Description** from the **Acquire** menu.
2. Click **Folder**.
3. Select the folder and folder location or create a new folder.
4. Click the **Select “(folder name)”** field at the bottom of the dialog box.
5. Click **File** in the **Parameter Description** window.
6. Type the Custom Prefix name (top line) that you wish to use for these files.
7. Click **OK**.
8. Type CEN in the **Sample ID** box in the **Parameter Description** window.
9. Close the **Parameter Description** window.

Acquiring CEN

1. Set the instrument flow rate to LO, turn the flow cytometer to RUN, mix the CEN sample, and install it on the cytometer's Sample Injection Port.
2. Make sure the cytometer is in Setup mode and click **Acquire** in the **Acquisition Control** window.
3. Make the following two adjustments. Click **Pause** and **Restart** in the **Acquisition Control** window as many times as needed. All adjustments are made in the Detectors/Amps window.
 - Viewing the FL2-A histogram, adjust the FL2-H photomultiplier tube (PMT) Voltage so the CEN singlets are in channel 200 ± 5 .
 - Viewing the FL2-W histogram, adjust only the FL2-W Amp Gain so the CEN singlets are in channel 200 ± 5 .

The channel number can be approximated by viewing the Mean column of the Histogram Statistics box.
4. Click **Pause** when done.
5. Click **Abort** in the **Acquisition Control** window.
6. Clear the **Setup** box in the **Acquisition Control** window.
7. Click **Acquire**.
8. After acquisition is complete, remove the CEN from the Sample Injection Port and place the cytometer in STANDBY.
9. Adjust markers (M) M1 and M2 on the first two peaks, singlets and doublets, of the FL2-A histogram so the peak falls within the respective marker.

10. Calculate the linearity using the mean channel number, located in the **Histogram Statistics** box, for marker 1 (M1) and the mean channel number for marker 2 (M2) using the following formula.

The linearity should be between 1.95 and 2.05. Locate the coefficient of variation (CV) of M1 in the FL2-A Histogram Statistics box. Check to see that the CV is less than or equal to 3.00%

$$\frac{\text{Mean M2}}{\text{Mean M1}} = \text{linearity}$$

11. Choose **Instrument Settings** from the **Cytometer** menu.
12. Click **Save**.
13. Type a name for the file, and specify a storage location.
14. Click **Save** in the directory dialog box.
15. Click **Done** in the **Instrument Settings** dialog box.

Acquiring CTN

To acquire CTN, the plot on page 2 of the DNA Experiment Document will need to be reformatted to an Acquisition -> Analysis plot.

1. Scroll to page 2 of the DNA Experiment Document.
2. Choose **Parameter Description** from the **Acquire** menu.
3. Type CTN in the **Sample ID** box.
4. Close the **Parameter Description** window.
5. Set the instrument flow rate to LO, turn the cytometer to RUN, and install the CTN sample on the cytometer's Sample Injection Port.
6. Click **Acquire**. A CTN sample (10,000 events) is collected. View the width versus area dot plot and display all 10,000 events. Verify there is good separation between the G₂+M portion of the singlet population and the doublets in FL2-W.
7. After acquisition is complete, remove the CTN tube from the Sample Injection Port and place the cytometer in STANDBY.
8. Save this new Experiment Document by choosing **Save** from the **File** menu.
9. Type a name for the document and specify a storage location.
10. Click **Save**.
11. Close the newly saved DNA Experiment Document.

The daily quality control of the instrument has been completed.

We recommend running a peripheral blood mononuclear cell (PBMC) sample or other diploid control to further adjust the instrument before acquiring samples. Prepare and stain the PBMCs the same way you would your specimens. For further information, see the *BD CellQuest™ DNA Experiment Document User's Guide*.

CV check of 2- μ m beads on FL2-Area

1. Open a new BD CellQuest™ Experiment Document or add a page to the DNA Experiment Document. For further information about Experiment Documents, refer to the *BD CellQuest™ Software User's Guide*.
2. Choose **Histogram Plot** from the **Plots** menu.
3. Change **Plot Source** to **Acquisition->Analysis**.
4. Select FL2-A in the **Parameter** field and click **OK**.
NOTE Enlarge the plot for easier viewing.
5. Choose **Histogram Stats** from the **Stats** menu.
6. Choose **Parameter Description** from the **Acquire** menu.
7. Select the folder where you will save the data and name the file.
8. Close the **Parameter Description** window.
9. Make sure the cytometer is connected and is in setup mode.
10. Set the instrument flow rate to LO, turn the cytometer to RUN, and install the 2- μ m beads on the cytometer's Sample Injection Port.
11. Click **Acquire**.
12. Adjust the FL2 Voltage to position the singlet peak at channel 200 $\pm$ 5.
NOTE The channel number can be estimated by viewing the Mean channel column in the Histogram Statistics box.
13. Click **Pause**, **Abort**, and **Setup** to remove the check mark.
14. Click **Acquire** to save the data file.
15. After acquisition is complete, remove the 2- μ m beads from the Sample Injection Port and place the cytometer in STANDBY.
16. Place a marker (M1) on the singlet peak and verify that the CV of M1 is less than or equal to 2.3%.

This Experiment Document can be saved and printed.

Procedure for Other BD Instruments

Refer to your cytometer user's guide and software reference manual for guidance on acquiring particles and analyzing the results.

Limitations

- Flow cytometric analysis of the DNA content requires that a linear relationship exists between PI fluorescence and the amount of DNA in the nucleus. This relationship is affected by a number of biological and physical variables, which include chromatin structure, the amount of double-stranded RNA in the nucleus, the fixation procedure, if any, and the pH and ionic strength of the staining solution. Therefore, the amount of fluorescence produced per milligram of DNA will be comparable only between the same tissues prepared in the same way.
- Exposure of the stained nuclei to light will reduce the fluorescence from the nuclei. Photo-bleaching may be prevented by keeping the stained nuclei in the dark until analysis.
- Large aggregates of nuclei can clog flow cytometers. Stream-in-air sorters can be vulnerable to clogging. To minimize this possibility, we recommend that the nuclei suspension be filtered through a 30- to 50- μ m nylon mesh prior to analysis.

- Care must be taken when using a different PI concentration or formulation than that included in this kit. If a different concentration or formulation of the dye solution must be used, stain the CEN and CTN with this solution and determine new PMT settings and target channels.
- The 2- μ m bead CV check procedure is intended for use in checking cytometer alignment or when the CV of the CEN or CTN is out of the specifications as stated. It is not intended for use in setup of the cytometer for DNA acquisition of specimens.
- The instrument must be thoroughly cleaned and regularly maintained as described in the instrument user's guide. This is especially true for sample preparations that contain free DNA, which can aggregate and have a high affinity for plastic tubing. It is also critical that no PI solution is left in the instrument when the next application or experiment is run.
- The flow rate should remain low (LO on BD FACSCalibur™ flow cytometers) for a more precise hydrodynamic focusing, yielding lower CVs.
- For use with other flow cytometers, all performance specifications will need to be determined for each individual case.

PERFORMANCE CHARACTERISTICS

CEN

The coefficient of variation (CV) of the CEN FL2-A singlet peak should be less than or equal to 3.00% for propidium iodide–stained nuclei when analyzed on a properly aligned flow cytometer. The acceptable range of linearity for the doublet/singlet ratio is 1.95 through 2.05. With the gains and detector voltages adjusted so the CEN FL2-A singlet peak is at channel 100 ± 9 , the human diploid peak should fall between channel 200 and 315, depending on the procedure used to obtain the diploid cells. Using BD CellQuest™ and the DNA Experiment Document on the Macintosh platform, the gains and detector voltages will need to be adjusted so the CEN FL2-A singlet peak is at channel 200 ± 5 . In this case, the human diploid control peak should fall between channel 500 and 600 and will need to be adjusted to channel 200 ± 5 .

NOTE Fluorescence of nuclei derived from paraffin-embedded tissues may fall outside this range.

CTN

A gate should be drawn to include the singlet population in the lower-left corner of the dot plot (but not debris found near the origin trailing up to the leading edge of the population). There should be a relatively clear visual separation between the G_2 +M singlets which follow the extending curve established by S-phase cells, and the G_0 / G_1 doublets, which will have a larger FL2-W signal along the horizontal axis. The number of dots displayed may need to be adjusted to make the separation more apparent to the eye. Tissue samples would require the FL2-A gate extending to the highest FL2-A channel in order to include all possible aneuploid cells.

2- μ m beads

The %CV of the beads can be checked on an FL2-A histogram with a target channel of 200. The FL2-A CV of the 2- μ m bead singlet peak should be less than or equal to 2.3% on a properly aligned flow cytometer. If this is not achieved, call the BD Biosciences Customer Support Center.

Troubleshooting

Problem	Possible Cause	Solution
Count rate too low.	• Instrument clogged. • Instrument out of calibration. • Sample too dilute.	• Perform daily maintenance. • Refer to the instrument user's guide. • Prepare new sample.
Unable to set PMT voltages or amplifier gains.	• Too many or too few CEN.	• Make a new stained CEN preparation. • Make a new stained CEN preparation or replace PI

Problem	Possible Cause	Solution
	- PI degradation. - CEN singlet gate too wide.	- stock solution. - Obtain a new gate.
Increased CV.	- PI or sample degradation. - Flow rate too high. - Air in fluidics.	- Make a new stained preparation. - Change the flow rate to low. - Drain and refill fluidics.
No doublet/singlet separation on FL2-W.	- FL2-W too low. - Sample or PI stain degradation.	- Check FL2-W gains. - Make a new stained CEN and CTN preparation or replace PI stock solution.

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.

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Patents and Trademarks

For US patents that may apply, see [bd.com/patents](https://www.bd.com/patents).

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