BECTON DICKINSON

DNA QC Particles
Cat. No. 349523

For Verification of Instrument Performance and Quality Control

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.

23-1889-09

1. INTENDED USE

For use in the setup and verification of the doublet discrimination function of FACSCalibur™, FACSort™, or FACScan™ flow cytometers equipped with a doublet discrimination module (DDM) for DNA analysis, and for monitoring daily instrument performance.

For use with FACS® brand, and 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.

2. 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 index[1] 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 being used for DNA analysis.

Most normal resting cells are diploid, while normal proliferating cells exist at various levels of ploidy. During the various phases of the cell cycle (G_0, G_1, S, G_2, and M), cell nuclei contain different amounts of DNA, characteristic of their stage of proliferation.

During the G_1 phase, cells undergo RNA and protein synthesis, maintaining diploidy (DNA content characteristic of two complete sets of chromosomes, 2 N). Subsequently, during the S phase, DNA synthesis occurs. Cells in this phase of the cell cycle contain amounts of DNA intermediate between G_1 and G_2. The S phase culminates in a doubling of DNA content, defined as G_2 tetraploid (4 N DNA content). During the G_2 phase, RNA and protein synthesis occurs, culminating in a mitosis phase (M). Based on DNA content alone, the M phase is indistinguishable from the G_2 phase. Once mitosis has occured, the resulting 2 N daughter cells either continue on to another cycle or enter a resting stage defined as G_0. This stage is indistinguishable from G_1 on the basis of the DNA content alone. Collectively, the replication cycle is described by G_0/G_1, S, and G_2+M phases.

The Becton Dickinson Immunocytometry Systems (BDIS) DNA Quality Control Particles provides a means for testing and documenting optimal performance on FACS brand 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.

3. PRINCIPLES OF THE PROCEDURE

CEN are prepared by a detergent treatment of chicken blood followed by fixation of the isolated nuclei in ethanol. These fixed nuclei may be used as instrument performance particles to set up flow cytometers such as the FACSCalibur, FACSort, FACSVantage™ SE, FACScan, FACStar^{PLUS}™, or FACStar™ 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 (Figure 2). When stained with propidium iodide, these aggregates result in four or more reference peaks in the fluorescence 2-area (FL2-A, Figure 3) or fluorescence 2-height (FL2-H, Figure 4) histograms as obtained using CellFIT™ software, on the Hewlett-Packard (HP) platform. The first four FL2-A histogram peaks represent the singlets, doublets, triplets, and quadruplets. 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 (see Figure 3). Comparable results are expected using CellQuest™ software and the DNA Experiment Document with the Macintosh® platform.

CTN are prepared by a detergent treatment 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 G_0/G_1 stage, and a smaller portion exists in the S and G_2+M stages. Doublets of the G_0/G_1 nuclei also exist and can be discriminated from true G_2+M singlets with the use of a DDM or pulse processing. The DDM enables doublets of cells in G_0/G_1 phase, and other aggregates, to be distinguished from single cells in G_2+M phase, thus allowing improved cell-cycle estimates of the percentage of cells in G_2+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 reducesCTN's ability to stain with PI.

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 Section 8, Limitations).

The 2-µm 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 is out of the specification stated in this insert, BD recommends running the 2-µm beads.

4. 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-µm fluorescent beads in buffer with gelatin and 0.1% azide
Vial D: 50 mL of 50-µg/mL solution of PI in buffer (25 tests using CEN and CTN)

Precautions

1. When stored at 2° to 8°C, the reagent is stable until the expiration date shown on the label. Do not use after the expiration date. Keep the reagent vials dry.
2. 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° to 8°C, since these particles are sensitive to thermal degradation.
3. CTN (vial B) tend to aggregate during storage and should be vigorously vortexed to separate these aggregates.
4. The 2-µm beads (vial C) must be protected from prolonged exposure to light.
5. Do not expose stained CEN or CTN to prolonged light during storage or incubation.
6. Incubation times or temperatures other than those specified may lead to erroneous results.
7. 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° to 8°C in the dark until analysis (maximum 12 hours).
8. **WARNING:** Propidium iodide is a suspected mutagen. Gloves and safety glasses should be worn when handling. Avoid contact with eyes, skin, and clothing. Avoid breathing vapors and wash surfaces thoroughly after handling. If contact occurs, flush immediately with water. Consult a physician if contact with eyes occurs.
9. **WARNING:** Formaldehyde is harmful by inhalation, in contact with skin, and if swallowed. It is irritating to eyes and skin. Exposure can cause cancer. Possible risks of irreversible effect. May cause sensitization by skin contact. Keep locked up and out of the reach of children. Keep away from food, drink, and animal feedingstuff. Wear suitable protective clothing and gloves. Even small amounts of formaldehyde can be fatal. If swallowed seek medical advice immediately and show this container or label. Dispose of according to federal, state, and local regulations.
10. **WARNING:** Sodium azide is harmful if swallowed. Keep out of reach of children. Keep away from food, drink, and animal feedingstuff. Wear suitable protective clothing. If swallowed, seek medical advice immediately and show this container or label. Contact with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions may develop.
11. **WARNING:** Thimerosal is a mercury compound. Exposure can cause reproductive toxicity. Harmful by inhalation, in contact with skin, and if swallowed. Danger of cumulative effects. Keep away from food, drink, and animal feedingstuff. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately. Dispose of according to federal, state, and local regulations.

5. INSTRUMENT

The above procedures using the DNA Quality Control Particles are designed for use on FACSCalibur, FACSort, and FACScan 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.

All the performance characteristics of this product were obtained using a Becton Dickinson FACScan flow cytometer on the HP platform equipped for fluorescence detection, forward and 90° angle light scatter, and doublet discrimination. The LO flow rate was selected. CEN, CTN, and 2-µm bead data were collected and analyzed with CellFIT software on the HP platform. CellQuest software and the DNA Experiment Document on the Macintosh platform may also be used to acquire DNA samples for analysis using an appropriate DNA analysis software package.

6. 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° to 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° to 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° to 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° to 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° to 8°C or in a covered ice bath until ready for analysis.

7. PROCEDURE

Reagent Provided

See Reagent Provided and Precautions in Section 4, Reagents.

Reagents and Materials Required but Not Provided

1. 12 x 75-mm disposable test tubes (Becton Dickinson Falcon® No. 2058, or equivalent)
2. Precision micropipettor, 1-mL capacity
3. Precision micropipettor, 50-µL capacity
3. Vortex mixer
4. Ice bath with cover
5. Disposable gloves
6. 1X phosphate-buffered saline (Dulbecco's, pH 7.2±0.2, 0.01 M),[7] calcium and magnesium free. Filter through a 0.2-µm filter before use. Store at 2° to 8°C.
7. 6 mL 12x75-mm Falcon tube with 35-µm strainer cap (BD Labware No. 35-2235)

Procedure for Setup on the Macintosh Platform

Before setting up your flow cytometer, turn on the flow cytometer, Macintosh computer system, and printer. For specific information, refer to the *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 may want to reposition the windows on the desktop.
NOTE: You can print the pages of your Experiment document by selecting Print from the File menu.

1. Choose Connect to Cytometer from the Acquire menu.
2. Choose Instrument Settings from the Cytometer menu.
3. Click Open.
4. Select the appropriate Instrument Settings file and click Open.
5. Click Set.
6. Click Done.
7. 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 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 you are 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. Uncheck 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 mean channel number for marker 2 (M2) using the following formula: $\frac{\text{Mean M2}}{\text{Mean M1}} = \text{linearity}$

    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%.
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 G_2+M portion of the singlet population and the doublets in FL2-W.
7. After acquisition is complete, remove CTN 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.

Becton Dickinson recommends 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, refer to the *CellQuest DNA Experiment Document User's Guide.*

CV Check of 2-µm Beads on FL2-Area
1. Open a new CellQuest Experiment Document or add a page to the DNA Experiment Document. For further information about Experiment documents refer to the *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.
   Select Folder, where you will save the data to, and name the file.
7. Close the Parameter Description window.
8. Make sure you are connected to the cytometer and are in setup mode.
9. Set the instrument flow rate to LO, turn the cytometer to RUN, and install the 2-µm beads on the cytometer's Sample Injection Port.
10. Click Acquire.
11. Adjust the FL2 Voltage to position the singlet peak at channel 200 ±5.
    NOTE: The channel number can be estimated by viewing the Mean channel column in the Histogram Statistics box.
12. Click Pause, Abort, and Setup to remove the check mark.
13. Click Acquire to save the data file to disk.
14. After acquisition is complete, remove the 2-µm beads from the Sample Injection Port and place the cytometer in STANDBY.
15. Place a marker (M1) on the singlet peak and verify that the CV of M1 is less than or equal to 2.3%.
16. This Experiment Document can be saved and printed.

Procedure for Setup on the HP Platform

CEN Setup
1. Enter the CellFIT program, select CellFIT from the MasterPage screen or from the HP Command Line, and execute:
   a) #11:/APP/DNA/CELLFIT, [Return], on a CONSORT 32 System,
   b) #ll:CELLFIT, [Return] or #12:CELLFIT, [Return], on a CONSORT 30 System.
2. At the CellFIT program main screen press [F1], SETUP.
3. Verify that each field labeled Particle Used is labeled CEN.
4. Verify the field labeled FL2-A Average is at the default value of 100. Make sure you set the cytometer to RUN and the fluidics to LO. Put the PI-stained CEN tube on the cytometer Sample Injection Port, then press [F1], ADJUST.
5. During the automatic PMT adjustment, verify the FL2-W and FL2-A measurements are being displayed. Allow the automatic setup to continue until it is complete. The following message will appear in the message box at the bottom of the screen: AUTODETECTORS HAVE BEEN SET.
6. Select [F3], CHECK CV. Verify that the CV of the singlet peak on FL2-A is less than or equal to 3.00% and that the ratio of doublets to singlets is between 1.95 and 2.05. Remove

the PI-stained CEN tube from the FACScan Sample Injection Port.
7. Print the Setup Report by pressing [F9]. Also obtain a histogram printout by pressing the [SHIFT] and [PRINT] keys simultaneously.

CTN Confirmation for Proper Cytometer DDM Function

1. Set up the cytometer with PI-stained CEN as in the Procedure for CEN Setup.
2. After printing the CEN Setup Report, select [F5], ACQSETUP.
3. Enter appropriate information into the various name fields. Enter appropriate file storage and file name information into the FILE STORAGE fields. Enter 10000 into the EVENTS field and put the PI-stained CTN tube on the cytometer Sample Injection Port.
4. Select [F5], ACQUIRE. This will start acquisition of a 10,000 event list-mode data file of ungated CTN.
5. After data acquistion is complete and the file is stored, remove the PI-stained CTN tube from the cytometer Sample Injection Port and select [F4], MODEL. Select SET GATES and set an FL2-W vs FL2-A singlet gate (Figure 5).
6. Mark the G_0/G_1 and G_2+M peaks on a singlet gated FL2-A histogram and analyze with the RFIT model.
7. Verify that the G_0/G_1 CV is less than or equal to 5.00%. Check the G_0/G_1, S, and G_2+M cell cycle percentages. For more information, refer to the *CTN Cell Cycle Statistics* insert included with this kit.

HP Procedure to Check 2-µm Bead CV on FL2-Area

1. Enter the CellFIT program. Select CellFIT from the MasterPage™ screen or from the HP Command line, and execute:
   a) #11:/APP/DNA/CELLFIT, [Return], on a CONSORT 32 System,
   b) #ll:CELLFIT, [Return] or #12:CELLFIT, [Return], on a CONSORT 30 System.
2. At the CellFIT program main screen, press [F1], SETUP.
3. Move the cursor to each field labeled Particle Used and type 2 µm beads, [Return].
4. Move the cursor to the field labeled FL2-A Average and type 200, [Return]. Put the 2-µm bead tube on the cytometer Sample Injection Port, then press [F1], ADJUST.
5. Immediately after the Acquisition Screen is drawn on the screen, press [F1], STOP ACQUISITION. Increase the FSC Gain on the cytometer to E01, then press [F3], RESTART.
6. During the PMT adjustment, verify FL2-W and FL2-A measurements are being displayed. Allow the automatic setup to continue until it is complete. The following message will appear in the message box at the bottom of the screen: AUTODETECTORS HAVE BEEN SET.
7. Select [F3], CHECK CV. Verify that the CV of the singlet peak on FL2-A is less than or equal to 2.3%. Remove the 2-µm bead tube from the cytometer Sample Injection Port.
8. Print the Setup Report by pressing [F9]. Also obtain a histogram printout by pressing the [SHIFT] and [PRINT] keys simultaneously.

8. LIMITATIONS

1. 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.
2. Exposure of the stained nuclei to light will reduce the fluorescence from the nuclei. Photobleaching may be prevented by keeping the stained nuclei in the dark until analysis.
3. Large aggregates of nuclei can clog flow cytometers. Stream-in-air sorters can be vulnerable to clogging. To minimize this possibility, it is suggested that the nuclei suspension be filtered through a 30- to 50-µm nylon mesh prior to analysis.
4. 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.

NOTE: The 2-µm 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 in this insert. It is not intended for use in setup of the cytometer for DNA acquisition of specimens.

5. 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 has 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.
6. The flow rate should remain low (LO on FACScan, FACSort, or FACSCalibur flow cytometers) for a more precise hydrodynamic focusing, yielding lower CVs.
7. For use with other flow cytometers, all performance specifications will need to be determined for each individual case.

9. PERFORMANCE CRITERIA

CEN

A typical CEN light-scatter dot plot is shown in Figure 1. Figure 2 illustrates a typical CEN dot plot of FL2-W versus FL2-A. Fluorescence 2-width (FL2-W) is a parameter that provides information about a particle's passage through the path of the laser. Figures 3 and 4 show typical CEN FL2-A and FL2-H histograms, respectively. The coefficient of variation (CV) of the CEN FL2-A singlet peak (Figure 3) should be less than or equal to 3.00% for propidium iodide–stained nuclei when analyzed on a properly aligned FACScan, FACSort, or FACSCalibur 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, with CellFIT version 2.0 or later (100 ±8 for CellFIT versions 1.0–1.2), the human diploid peak should fall between channel 200 and 315, depending on the procedure used to obtain the diploid cells. Using 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.

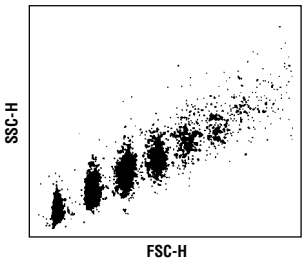

Figure 1. Representative of forward vs 90° scatter of CEN acquired on a FACScan flow cytometer using CellFIT software.

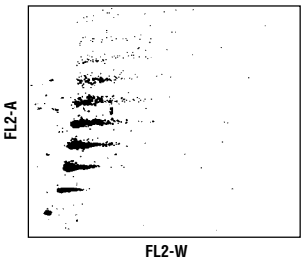

Figure 2. Demonstration of CEN aggregates on the basis of FL2-W vs FL2-A.

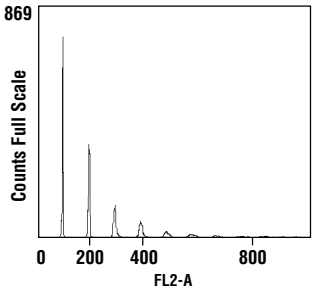

Figure 3. FL2-Area histogram of PI-stained CEN demonstrating resolution and linearity.

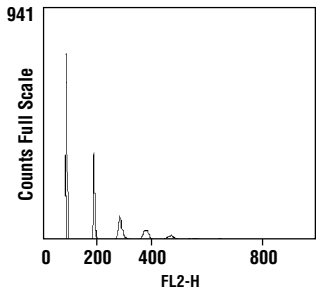

Figure 4. The same PI-stained CEN populations shown as FL2-height measurement.

CTN

Figure 5 shows a CTN dot plot demonstrating gating around the singlet population on the basis of FL2-W versus FL2-A. The 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. (In this figure, the gate is drawn to enclose the upper limit of the G_2+M singlet population because CTN are known not to be polyploid. Tissue samples would require the FL2-A gate extending to the highest FL2-A channel in order to include all possible aneuploid cells.)

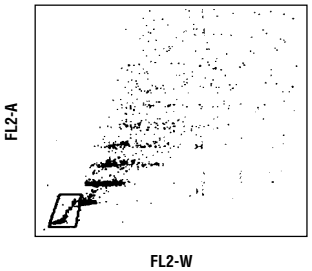

Figure 5. FL2-W vs FL2-A CTN dot plot demonstrating gate drawn to include only singlets.

Figure 6 displays forward scatter (FSC) versus FL2-A, and illustrates the difficulty in distinguishing singlets from doublets on the basis of light scatter. Figure 7 displays the single parameter FL2-A histogram of the CTN data file as previously gated in Figure 5, showing distinct G_0/G_1, S, and G_2+M cell-cycle phases. The CV of the CTN G_0/G_1 singlet peak in the FL2-A histogram should be less than or equal to 5.00% when analyzed in the RFIT model with CellFIT software.

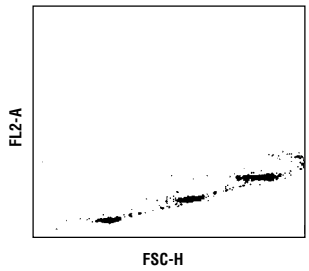

Figure 6. Forward scatter vs FL2-A CTN. Note this display cannot be easily used to identify doublets on the basis of forward scatter.

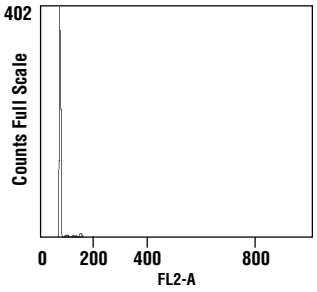

Figure 7. This histogram of CTN FL2-A shows G_0/G_1, S and G_2+M phases. Data has been gated as shown in Figure 5.

2-µm Beads

Figure 8 shows representative FL2-A data of the 2-µm beads. The %CV of the beads was checked on an FL2-A histogram with a target channel of 200. The FL2-A CV of the 2-µm bead singlet peak should be less than or equal to 2.3% on a properly aligned FACScan flow cytometer. If this is not achieved, call your nearest Customer Support Center.

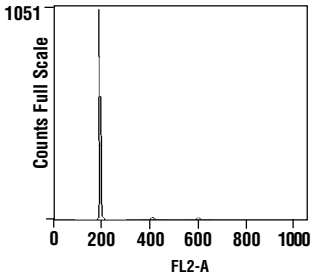

Figure 8. FL2-A histogram of 2-µm fluorescent beads, acquired and analyzed using CellFIT software.

10. TROUBLESHOOTING

Problem	Possible Cause	Solution
Count rate too low.	1. Instrument clogged. 2. Instrument out of calibration. 3. Sample too dilute.	1. Perform daily maintenance. 2. Refer to the instrument user's guide. 3. Prepare new sample.
Unable to set PMT voltages or amplifier gains.	1. Too many or too few CEN. 2. PI degradation. 3. CEN singlet gate too wide.	1. Make a new stained CEN preparation. 2. Make a new stained CEN preparation or replace PI stock solution. 3. Repeat Adjust Screen to obtain a new gate.
Increased CV.	1. PI or sample degradation. 2. Flow rate switched on HI. 3. Air in fluidics.	1. Make a new stained preparation. 2. Change Flow switch to LO. 3. Drain and refill fluidics.
No doublet/singlet separation on FL2-W.	1. FL2-W too low. 2. Sample or PI stain degradation.	1. Check FL2-W gains. 2. Make a new stained CEN and CTN preparation or replace PI stock solution.

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