PC7 (770/488)
SETUP KIT

6607121

PN 4299504-C

BECKMAN COULTER™

FLOW CYTOMETER ALIGNMENT VERIFICATION FLUOROSPHERES

FLOW CYTOMETER DETECTOR STANDARDIZATION FLUOROSPHERES

INTENDED USE

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

PRODUCT DESCRIPTION

The PC7 (770/488) Setup Kit is used for daily verification of a flow cytometer's optical alignment and fluidic stability and as an aid in standardizing the detectors on a flow cytometer for the quantitative analysis of human leukocytes. The kit is comprised of two vials: one vial of FLOW-CHECK™ 770 fluorospheres and one vial of FLOW-SET™ 770 fluorospheres.

SUMMARY AND EXPLANATION

In flow cytometric analysis, instrument setup is required to ensure consistent analysis of samples. Instrument setup involves alignment verification of optical detectors, verification of fluidic stability, standardization of instrument detector settings, and establishment of color compensation settings. Instrument setup is performed for all light scatter and fluorescence parameters. The PC7 (770/488) Setup Kit is used to set up flow cytometers equipped with both an Argon Ion laser (488 nm laser line) and detectors for the measurement of CY7 emission. The kit is intended to verify the optical alignment and standardize the fluorescence detectors for PE-CY7 with a 755 (or equivalent) band pass filter. Mixing aliquots of FLOW-CHECK 770 fluorospheres or FLOW-SET 770 fluorospheres with other fluorosphere suspensions such as FLOW-CHECK or FLOW-SET fluorospheres allows for multicolor instrument setup.

Optical Alignment and Fluidic Stability Verification

The use of uniform fluorospheres to verify optical alignment has been well established.[1-3] FLOW-CHECK 770 fluorospheres is a suspension of fluorospheres with uniform and stable size and intensity. The uniformity of these product parameters allows for adjustment and/or verification of the alignment of the optical and fluidic systems of flow cytometers. Optical alignment and fluidic stability can be verified by calculating the Coefficient of Variation (CV) or Half Peak Coefficient of Variation (HPCV) and mode intensity of a sample population.

Optical Detector Standardization

It is important that light scatter and fluorescence parameters on a flow cytometer are standardized to provide optimal instrument performance on a daily basis. Instrument standardization should be performed with the same detector settings used to run test samples.[1] Each laboratory should determine optimum instrument settings for their own instruments and establish their own daily values.

The use of fluorospheres to standardize light scatter intensity and optimize hydrodynamic focusing is widely accepted.[1-3] These instrument parameters can be standardized by determining the values of FS, SS or Log SS, and fluorescence intensity when the fluorospheres are tested using laboratory and application specific instrument settings. Instrument performance can be monitored daily by measuring the instrument settings needed to obtain the values for each of the desired parameters of fluorospheres.

FLOW-SET 770 fluorospheres are excited using an Argon (Ar) Ion laser and are intended to standardize the PE-CY7 channel (750 nm and above).

REAGENTS

PC7 (770/488) Setup Kit, PN 6607121, contains the following:
FLOW-CHECK 770 Fluorospheres - 1 x 10 mL vial
FLOW-SET 770 Fluorospheres - 1 x 10 mL vial

REAGENT CONTENTS

FLOW-CHECK 770 fluorospheres consist of 7 µm (nominal diameter) polystyrene fluorospheres. FLOW-SET 770 fluorospheres consist of 6 µm (nominal diameter) polystyrene fluorospheres. FLOW-CHECK 770 and FLOW-SET 770 fluorospheres are suspended in an aqueous suspension medium containing surfactants, and 2 mM NaN_3 at 1 x 10^6 fluorospheres/mL (nominal concentration). Each fluorosphere contains a dye that has a fluorescence emission from 700 nm to 800 nm when excited at 488 nm.

STATEMENT OF WARNINGS

1. These reagents contain 2 mM sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. These products should only be used in their suspending medium. Addition of organic solvents or high ionic strength solutions may irreversibly swell or aggregate fluorospheres.
3. Ensure the fluorospheres are completely resuspended before use. Fluorospheres may settle over extended periods of time.
4. Do not use the fluorospheres beyond the expiration date on the vial label.
5. Minimize exposure of reagents to light during storage or use.
6. Use Good Laboratory Practice (GLP) when handling this reagent.

STORAGE CONDITIONS AND STABILITY

These reagents are stable to the expiration date on the vial label when stored at 2-8°C. Do not freeze. Minimize exposure to light. Opened vials must be refrigerated after use and are stable to the expiration date on the vial label. Fluorospheres dispensed into test tubes must be discarded daily after use.

EVIDENCE OF DETERIORATION

Inability to obtain expected results may indicate product instability or deterioration. Product deterioration may be indicated by secondary fluorescent populations in excess of 15% of the total flourescent population or bimodal distribution where the peak height of the second peak exceeds 10% of the main peak, when only using one laser (488 nm).

ALIGNMENT VERIFICATION PROCEDURE

Reagent Preparation

Proper mixing of the individual fluorosphere components is required prior to use. FLOW-CHECK 770 fluorospheres should be used in conjunction with FLOW-CHECK fluorospheres and optionally with the APC (675/633) Setup Kit to set up additional multicolor/multilaser applications. The individual fluorosphere suspensions in these kits are identified by their FS intensity. The fluorescence histograms of each fluorescence parameter are gated on the respective FS singlet population. The following matrix provides examples of bead mixtures that can be used for various applications.

Application	FLOW-CHECK	FLOW-CHECK 770 (optional)	FLOW-CHECK 675
FS-FITC-PE-ECD-PC5	✓		
FS-FITC-PE-ECD-PC5-PC7*	✓	✓	
FS-FITC-PE-ECD-APC*	✓		✓
FS-FITC-PE-ECD-APC-PC7**	✓	✓	✓

* Bead mixtures containing FLOW-CHECK fluorospheres and another bead should be at a ratio of 2:1. . Add approximately 0.4 mL (10-15 drops) of FLOW-CHECK fluorospheres and approximately 0.2 mL (5-8 drops) of FLOW-CHECK 770 or FLOW-CHECK 675 fluorospheres to the test tube.

** Bead mixtures containing FLOW-CHECK fluorospheres and two other beads should be at a ratio of 2:1:1. Add approximately 0.4 mL (10-15 drops) of FLOW-CHECK fluorospheres and approximately 0.2 mL (5-8 drops) of FLOW-CHECK 770 and approximately 0.2 mL (5-8 drops) of FLOW-CHECK 675 fluorospheres to the test tube.

MATERIAL SUPPLIED

FLOW-CHECK 770 Fluorospheres - 1 x 10^6 fluorospheres/mL

MATERIALS REQUIRED BUT NOT SUPPLIED

FLOW-CHECK Fluorospheres, PN 6605359
APC (675/633) Setup Kit, PN 6607120 (Optional for 5 color 2 laser applications)
Appropriately sized test tubes
Flow Cytometer

Procedure For Establishing Values For Daily Use

1. Use the normal filter set recommended by the manufacturer for detecting the appropriate fluorescence parameters (refer to Operator Manuals).
2. Create a fluorosphere test protocol containing a single parameter ungated histogram of FS and single parameter histograms for each desired fluorescence parameter. Create a gate for each of the fluorospheres in the fluorosphere mixture to be utilized in the FS histogram. Create a second FS single parameter histogram gated on FLOW-CHECK fluorospheres. Assign the gates to the appropriate fluorescence histogram. The FLOW-CHECK 770 fluorospheres gate should be assigned to the histogram where PE-CY7 will be measured. All other histograms should be gated on FLOW-CHECK fluorospheres (see Figure 1). Set the color compensation to zero percent (0%) for all fluorescence compensations. Set the sample rate to "Low." Set a minimum stop count of 5,000 events on the FS histogram gated on FLOW-CHECK fluorospheres.
3. Vortex and aspirate the fluorosphere mixture as prepared in the Reagent Preparation section until no sediment appears in the bottom of the 12 x 75 mm test tube.
4. If necessary, adjust the FS detector settings so that the highest intensity FS signal (FLOW-CHECK fluorospheres) is in the center of the histogram. FLOW-CHECK 770 fluorospheres will be the population lower than the FLOW-CHECK fluorospheres. Establish the appropriate gates for FLOW-CHECK fluorospheres (gated to FS, FL1-FL4) and FLOW-CHECK 770 fluorospheres (FL5). See Figure 1.
5. Adjust the fluorescence detectors to place the gated populations to the center of their respective histograms. Create regions in each of the gated histograms that encompass the gated populations.
6. If your instrument alignment is user adjusted, perform the following, otherwise proceed to step 8.
   a. Optimize the light scatter signal (refer to Instrument Manuals).
   b. Optimize the fluorescence signal(s) (refer to Instrument Manuals).

c. If necessary, readjust the FS detector settings so that the highest intensity population is mid-scale in the histogram.
d. If necessary, readjust the detector settings on the linear fluorescence signals such that each peak is about mid-scale in the histogram.

7. Record the HPCV and Mode (Peak Position) for the gated FS histogram, and each of the appropriate fluorescence parameters in a log (see Figure 2).

   NOTE: For instruments on which mode or peak position is not readily available, mean intensity may be used if the position of the gates and regions remain constant day to day.

8. Repeat steps 1 - 7 until at least 20 data points have been collected at various time intervals following instrument warm up. Data should be collected over 5 days. After the data points have been collected and recorded, calculate and record the Average, Standard Deviation (SD), and ±2 SD of the HPCV data in Figure 2. Calculate the Average ±5% of the MODE in Figure 2. These values represent the expected ranges for HPCV and mode for your laboratory.
9. Compare your Average values to ensure that they are below the acceptance limits for HPCV for the appropriate fluorosphere/parameter for each lot of FLOW-CHECK and FLOW-CHECK 770 fluorospheres. If the values are above the acceptance limits, refer to the TROUBLESHOOTING Section, or where appropriate, adjust the alignment again.
10. Create a Levey-Jennings plot for the HPCV of each desired parameter using the mean and ±2 SD range as calculated in step 9 (see Figure 3).
11. Repeat steps 1-10 any time significant changes are made to light scatter or fluorescence functions such as changes in filter configurations.
12. When changing lots of FLOW-CHECK or FLOW-CHECK 770 fluorospheres, repeat the Procedure for Establishing Values for Daily Use.

Procedure For Daily Verification of Alignment and Fluidics

1. Use the normal filter set recommended by the manufacturer for detecting the appropriate fluorescence parameters (refer to Operator Manuals).
2. Select the fluorosphere test protocol (refer to step 2 in Procedure for Establishing Values for Daily Use).
3. Vortex and aspirate the fluorosphere mixture prepared in the Reagent Preparation section until no sediment appears in the bottom of the 12 x 75 mm test tube.
4. If necessary, adjust the appropriate detector settings to place each peak within the mode range established (refer to the Procedure For Establishing Values For Daily Use).
5. Record the HPCV and Mode values for each desired parameter (see Figure 4).

   NOTE: For instruments on which mode or peak position is not readily available, mean intensity may be used if the position of the gates and regions remain constant day to day.

6. Record the HPCV values for each parameter on their respective Levey-Jennings graph (see Figure 3).
7. Ninety-five percent (95%) of values should fall within the ±2 SD range for each parameter. If values drift outside this range, refer to the TROUBLESHOOTING Section.

LIMITATIONS

1. Fluorospheres analyzed at increased flow rates may exhibit wider population distributions and a wider HPCV.
2. Ensure that the instrument is properly warmed up and that the ambient temperature is within the range specified in the Instrument Manuals.
3. Day to day analysis of fluorospheres should be conducted at the same mode intensities determined in the Procedure for Establishing Values For Daily Use.
4. The mixture of FLOW-CHECK in conjunction with FLOW-CHECK 675 and/or FLOW-CHECK 770 should not be used to set up IVD applications.
5. FLOW-CHECK 770 Fluorospheres will have a different fluorescence intensity, fluorescence distribution and HPCV when analyzed using both an Argon laser and HeNe laser as compared to an Argon laser alone.

TROUBLESHOOTING

1. Ensure that the fluorosphere sample has not been diluted or contaminated. Dilution of the fluorosphere mixture beyond the ratios described in the Reagent Preparation section may increase the HPCV values recovered.
2. Ensure that the fluorosphere mixture has been adequately mixed so that no sediment is observed.
3. Ensure the cap of the sheath tank is secure and not leaking.
4. Check for excessive bubbles in the sheath filter. If a clog or bubble is suspected, flush or prime the sample line.
5. If the FL4 HPCV is above 2.5% when analyzing a mixture containing FLOW-CHECK 675 Fluorospheres, refer to the instrument manual instructions for adjusting the HeNe laser.
6. Refer to the Operator Manuals for additional troubleshooting steps.

EXPECTED RESULTS

Expected results were determined using a Cytomics FC 500 flow cytometer equipped with RXP Software, recommended filters for five color analysis, and both Ar Ion and HeNe lasers. HPCV values for FLOW-CHECK fluorospheres should be less than 2% for FS, FL1-FL3, and FL4 (Ar excitation). HPCV values for FLOW-CHECK 770 fluorospheres should be less than 4%. A COULTER® MULTISIZER™/ACCUCOMP® system was used for particle size analysis and counting.

Each laboratory must establish its own expected ranges based on its instrument, instrument setup, and operating conditions. Expected ranges may vary slightly due to instrument differences such as optical filters, laser power, laser emission wavelength, laser mode, flow cell type, sample delivery rate, and statistical analysis package. Therefore, a laboratory quality assurance program will require replicate sample determinations and calculation of mean and standard deviation statistics for all desired parameters.

DETECTOR INTENSITY STANDARDIZATION PROCEDURE

Reagent Preparation

Proper mixing of the individual fluorosphere components is required prior to use. FLOW-SET 770 fluorospheres must be mixed with FLOW-SET fluorospheres to perform detector intensity standardization when using an Ar Ion laser. For 5 color applications with 2 lasers, add FLOW-SET 675 fluorospheres to the mixture. The individual fluorosphere suspensions in these kits are identified by their FS intensity. The fluorescence histograms of each fluorescence parameter are gated on the respective FS singlet population. The following matrix provides examples of bead mixtures that can be used for various applications.

Application	FLOW-SET	FLOW-SET 770 (optional)	FLOW-SET 675
FS-SS-FITC-PE-ECD-PC5	✓		
FS-SS-FITC-PE-ECD-PC5-PC7*	✓	✓	
FS-SS-FITC-PE-ECD-APC*	✓		✓
FS-SS-FITC-PE-ECD-APC-PC7**	✓	✓	✓

*Bead mixtures containing FLOW-SET fluorospheres and another bead should be at a ratio of 2:1.
Add approximately 0.4 mL (10-15 drops) of FLOW-SET fluorospheres and approximately 0.2 mL
(5-8 drops) of FLOW-SET 770 or FLOW-SET 675 fluorospheres to the test tube.

**Bead mixtures containing FLOW-SET fluorospheres and two other beads should be at a ratio of
2:1:1. Add approximately 0.4 mL (10-15 drops) of FLOW-SET fluorospheres and approximately
0.2 mL (5-8 drops) of FLOW-SET 770 and approximately 0.2 mL (5-8 drops) of FLOW-SET 675 fluorospheres to the test tube.

MATERIALS SUPPLIED

FLOW-SET 770 Fluorospheres - 1 x 10^6 fluorospheres/mL

MATERIALS REQUIRED BUT NOT SUPPLIED

FLOW-SET Fluorospheres, PN 6607007
APC (675/633) Setup Kit, PN 6607120 (Optional for 5 color 2 laser applications)
Appropriately sized test tubes
Flow Cytometer

The table below summarizes procedures for three typical laboratory scenarios concerning instrument standardization.

SUMMARY TABLE OF PROCEDURES

NOTE: Number denotes suggested order of performance.

	Laboratory Scenario		
Procedure For	No Standardization Protocol in Use	Established Procedure in Use (Fluorescence Parameters Only)*	Lot to Lot Comparison
Establishing Fluorescence and/or Light Scatter Target Ranges	✓①	✓①	
Establishing Instrument HV/Gain Ranges	✓②	✓②	
Daily Instrument Standardization	✓③	✓③	✓②
Lot to Lot Verification	✓④	✓④	✓①

* This represents laboratories that need to add light scatter standardization to current laboratory-established standardization procedures

Procedure for Establishing Fluorescence and/or Light Scatter Target Ranges

NOTE: Use this procedure to: (a) establish target ranges for fluorescence and light scatter parameters when either the flow cytometer or the reagent application has not been standardized and, (b) add CD45 gating to a current laboratory-established fluorescence standardization protocol.

1. Verify that the flow cytometer is optimally aligned (refer to the Procedure for Performing Alignment Verification).
2. Use the filter set recommended by the manufacturer for detecting the fluorescence parameters to be analyzed (refer to the Operator Manuals or the reagent package insert).
3.
   a. **For fluorescence and light scatter ranges:**
   Using the appropriate test protocol, run a sample stained with a representative isotype control and adjust FS and SS or Log SS high voltages and gains to optimize the resolution of the leukocyte clusters. Adjust the appropriate log fluorescence high voltages (HV) to position the negative fluorescence at the end of the first decade. Record the instrument settings for FS, SS and fluorescence parameters (see Figure 5).
   b. **For CD45 gating:**
   Using the appropriate test protocol, run a sample stained with a monoclonal antibody reagent containing CD45. Adjust the SS and CD45 fluorescence high voltages and gains to optimize the resolution of all leukocyte clusters. Record the

instrument settings for SS and CD45 parameters (see Figure 5). Current laboratory-established values for fluorescence high voltages should also be recorded.

4. Create a fluorosphere target-setting protocol of single parameter histograms for each desired light scatter and log fluorescence parameter. The protocol should contain a single parameter ungated histogram of FS, and single parameter histograms for SS and each desired fluorescence parameter. Create a gate for each of the fluorospheres in the fluorosphere mixture to be utilized in the FS histogram. Assign the gates to the appropriate fluorescence histogram(s). The FLOW-SET 770 fluorospheres gate should be assigned to the histogram where PE-CY7 will be measured. SS and Log FL1-Log FL3 histograms should be gated on FLOW-SET fluorospheres (see Figure 6). Set color compensation to zero percent (0%) for all fluorescence compensations. Set laser power and detector settings to the values which were established in step 3. Set the sample rate to "Low." Set a stop count of 5,000 events on the Log FL1 histogram gated on FLOW-SET fluorospheres. Set the FS discriminator to a value of 50.
5. Vortex and aspirate the fluorosphere mixture as prepared in the Reagent Preparation section until no sediment appears in the bottom of the 12 x 75 mm test tube.
6. Figure 6 illustrates an analysis using the three Fluorosphere mixture. If necessary adjust the FS discriminator to a level which displays the number of populations in the fluosphere mixture. Ensure that cursors are placed across the population peaks for all parameters.
7. Record the population mode values (Peak Position) for all desired parameters (see Figure 5).

   NOTE: For instruments on which mode or peak position is not readily available, mean intensity may be used if the position of the gates and regions remain constant day to day.

8. After the appropriate number of representative samples have been collected and recorded, calculate and record the average Mode (Peak Position) and determine target ranges for each desired parameter.
9. If your system has automated capability for detector standardization, refer to software documentation for proper protocol setup.
10. Repeat steps 1-8 for each sample preparation method, each new application requiring different instrument settings, and any time significant changes are made to light scatter or fluorescence signals (for example, photomultiplier tube replacement or laser alignment).
11. After establishing target ranges for fluorescence and light scatter parameters, perform the Procedure For Establishing Instrument High Voltage (HV) Ranges.

Procedure For Establishing Instrument High Voltage (HV) Ranges

NOTE: Use this procedure after target ranges for fluorescence and light scatter parameters have been established.

1. Verify that the flow cytometer is optimally aligned (refer to the Procedure for Performing Alignment Verification).
2. Use the filter set recommended by the manufacturer for detecting the fluorescence parameters to be analyzed (refer to the Operator Manuals or the reagent package insert).
3. Select the fluorosphere target setting protocol for analysis (refer to step 4 in the Procedure For Establishing Fluorescence And/Or Light Scatter Target Ranges).
4. Vortex and aspirate the fluorosphere mixture prepared in the Reagent Preparation section until no sediment appears in the bottom of the 12 x 75 mm test tube.
5. Utilize protocol previously created in step 4 in the Procedure for Establishing Fluorescence and/or Light Scatter Target Ranges section. Figure 6 illustrates an analysis using the three Fluorosphere mixture. If necessary adjust the FS discriminator to a level which displays the number of populations in the fluorosphere mixture. Ensure that cursors are placed across the population peaks for all parameters.
6. Adjust instrument settings to place each fluorosphere Mode (Peak Position) within the target range determined as your laboratory reference range (refer to the Procedure For Establishing Fluorescence And/Or Light Scatter Target Ranges).
7. Record the mode value and HV for each parameter and record gains for light scatter (see Figure 7).
8. Continue to run the fluorosphere mixture using the fluorosphere target setting protocol until 20 data points have been collected. Collect data at various time intervals following instrument warm up and alignment verification over at least 5 days (being sure to record the settings as described in step 8). After 20 data points have been collected and recorded, calculate and record the Average, ±2 SD range or ±1% range (whichever is greater) for HV for each parameter.
9. Use the Average ±2 SD range or ±1% range to create a Levey-Jennings chart for each desired parameter (see Figure 8).
10. Ninety-five percent (95%) of values should fall within the ±2 SD range or ±1% range (whichever is greater) of the HV ranges for each parameter.
11. After establishing instrument HV ranges, perform the Procedure For Daily Instrument Standardization.

Procedure For Daily Instrument Standardization

1. Verify that the flow cytometer is optimally aligned (refer to the Procedure for Performing Alignment Verification).
2. Use the filter set recommended by the manufacturer for detecting the fluorescence parameters to be analyzed (refer to the Operator Manuals or the reagent package insert).
3. Select the fluorosphere target setting protocol for analysis (refer to step 4 in the Procedure For Establishing Fluorescence And/Or Light Scatter Target Ranges).
4. Vortex and aspirate the fluorosphere mixture as prepared in the Reagent Preparation section until no sediment appears in the bottom of the 12 x 75 mm test tube.
5. Adjust instrument settings to place each fluorosphere peak within the target range determined as your laboratory reference range (refer to the Procedure For Establishing Fluorescence And/Or Light Scatter Target Ranges).
6. Record the mode (Peak Position) and HV for each parameter and light scatter gains in a daily log (see Figure 9).
7. Plot the HV and Gain values for each desired parameter on its respective Levey-Jennings chart (see Figure 8).
8. Ninety-five percent (95%) of values should fall within the ±2 SD range or ±1% range (whichever is greater) of the HV ranges for each parameter. If values drift outside this range, refer to the TROUBLESHOOTING section.

Procedure For Lot-To-Lot Verification

NOTE: : Use this procedure when changing lots of FLOW-SET, FLOW-SET 770, and FLOW-SET 675 fluorospheres. Run the new and the old lots in parallel, according to the laboratory's established procedures, to determine the average Mode (Peak Position) and target ranges for the new lot.

1. Verify that the flow cytometer is optimally aligned (refer to the Procedure for Performing Alignment Verification).
2. Use the filter set recommended by the manufacturer for detecting the fluorescence parameters to be analyzed (refer to the Operator Manuals or the reagent package insert).
3. Select the standardization protocol currently in use in the laboratory.
4. Prepare the desired fluorosphere mixture with new lot(s) of fluorospheres in a 12 x 75 mm test tube as described in the Reagent Preparation section.
5. Vortex and aspirate the fluorosphere suspension in the test tube until no sediment remains on the bottom of the tube.
6. Using the fluorosphere mixture with the current lots of fluorospheres, perform the daily standardization procedure. Record the Mode (Peak Position) and HV for each parameter and record gains for light scatter in the laboratory's current daily logbook.
7.
   a. **For nonautostandardization protocols:**
      Run the new fluorosphere mixture with the new lot of fluorospheres using the same settings as step 3 above. Ensure that the cursors are placed around the population peaks for all desired parameters.
   b. **For autostandardization protocols:**
      Create a copy of the current autostandardization protocol. The copy should have the autostandardization function disabled. Using the new protocol, run the new fluorosphere lot(s) at the same settings as in step 3 above. Ensure that the cursors are placed around the population peaks for all desired parameters.
8. Record the Mode (Peak Position) for all desired parameters (see Figure 7).
9. After the appropriate number of repetitions have been completed and recorded, calculate and record the average Mode intensity and determine target ranges for each parameter.
10. If your system has automated capability for detector standardization, refer to software documentation for proper protocol setup.
11. Transfer the new target channels to the fluorosphere target setting protocol.
12. Use the new target ranges when performing the Procedure For Daily Instrument Standardization.

LIMITATIONS

1. Instrument settings vary according to the sample preparation method used and should be set accordingly. FLOW-SET 770 fluorospheres may not be appropriate for some sample preparation methods. Each laboratory should determine its own reference ranges for each instrument, each sample preparation method and each fluorescent dye used.
2. Inconsistent values may occur if fluorospheres settle in the sample lines of the flow cytometer.
3. The mixture of FLOW-SET fluorospheres in conjunction with FLOW-SET 770 and/or FLOW-SET 675 fluorospheres should not be used to set up IVD applications.

TROUBLESHOOTING

1. Ensure that the sample has not been diluted or contaminated.
2. Ensure that the fluorosphere vials and mixtures have been adequately mixed so that no sediment is seen.
3. Ensure that the cap of the sheath tank is secure and not leaking.
4. Check for excessive bubbles in the sheath filter. If a clog or bubble is suspected, flush, or prime the sample line.
5. Refer to the Operator Manuals for additional troubleshooting steps.
6. FLOW-SET Fluorospheres will have a different fluorescence intensity, fluorescence distribution, and HPCV when analyzed using both an Argon and HeNe laser as compared to an Argon laser alone.

REFERENCES

1. National Committee for Clinical Laboratory Standards. Clinical applications of flow cytometry: Quality assurance and immunophenotyping of peripheral blood lymphocytes; Tentative Guideline. 1992. NCCLS Document H42-T, pp. 14-21, 26-27.
2. Guideline for flow cytometric immunophenotyping: A report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. 1993. Cytometry, 14:702-715.
3. Revised guidelines for performing CD4+ T-Cell determinations in persons with human immunodeficiency virus (HIV). January 10, 1997. MMWR, 46:12-13.

PRODUCT AVAILABILITY

PC7 (770/488) Setup Kit, PN 6607121, contains the following:

FLOW-CHECK 770 Fluorospheres……..1 x 10^6 fluorospheres/mL vial
FLOW-SET 770 Fluorospheres……..1 x 10^6 fluorospheres/mL vial

For additional information in the USA, call 800-526-7694. Outside the USA, contact your local Beckman Coulter Representative.

TRADEMARKS

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Printed in USA
Made in USA

Figure 1: Alignment Verification Protocol for Five Color with Two lasers collected on a Cytomics FC 500 with RXP Software

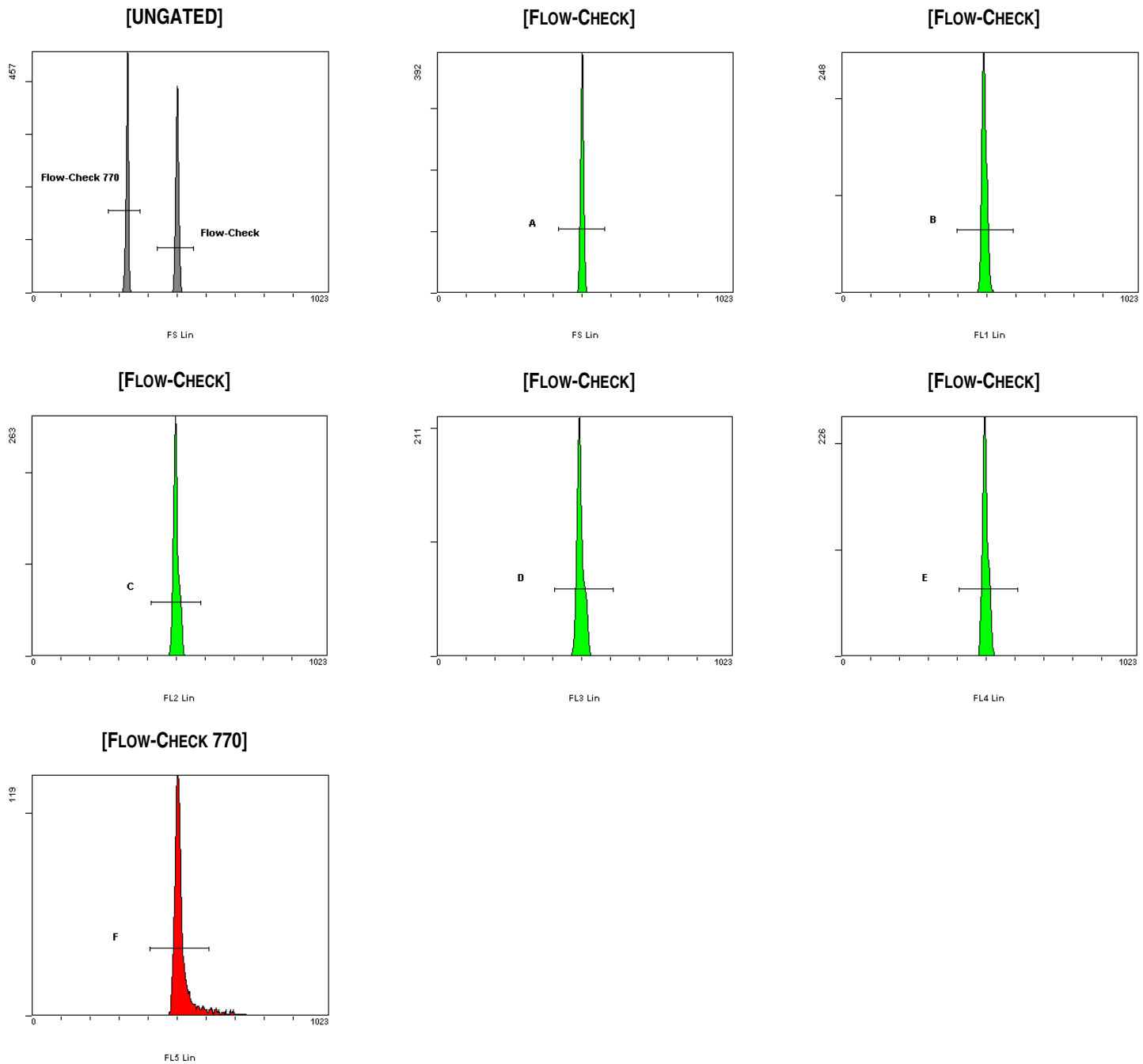

Figure 2: Example of a Table to Record HPCV and Mode Values when Establishing Values for Daily Use.

ESTABLISHING PEAK POSITION AND HPCV TARGET RANGES

FLOW-CHECK ™ Fluorospheres

Lot Number _____

Expiration Date _____

	FS		FL1		FL2		FL3		FL4		FL5		
Run	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Tech/Date
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													
Mean													
SD													
+2SD													
-2SD													

Serial No. _____

Lab. _____

Cytomics FC 500

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Figure 3: Example of a Levey-Jennings Chart for Plotting Daily HPCV Values.

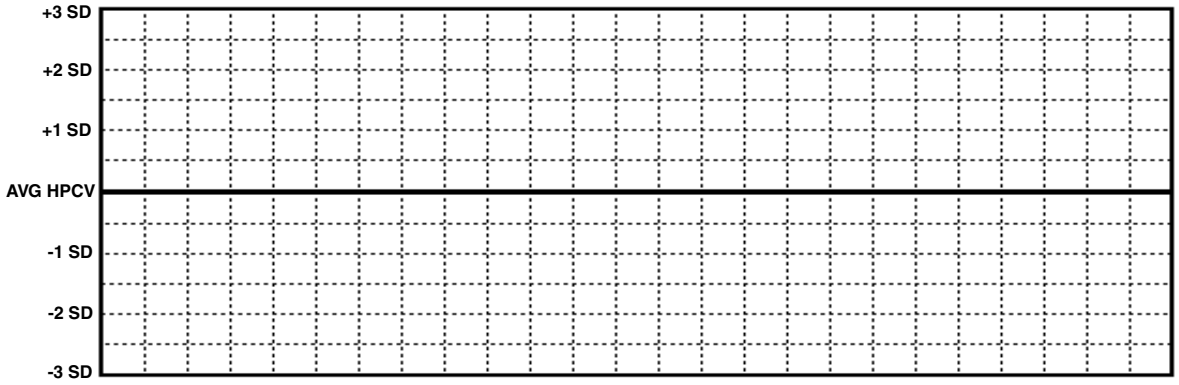

Figure 4: Example of a Table to Record HPCV and Mode Values for Daily Verification of Alignment and Fluidics.

DAILY LOG FOR INSTRUMENT VERIFICATION OF ALIGNMENT AND FLUIDICS

FLOW-CHECK™ Fluorospheres

Lot Number _____

Expiration Date _____

Target Range	FS		FL1		FL2		FL3		FL4		FL5		
Run	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Tech/Date
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
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Serial No. _____

Lab. _____

Cytomics FC 500

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Figure 5: Example of a Table to Record Target Intensity Standardization Mode, High Voltage and Gain Settings when Establishing Fluorescence and/or Light Scatter Target Ranges.

	FS			SS			FL1		FL2		FL3		FL4		FL5	
Run	Mode	HV*	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Mode	HV
1																
2																
3																
Average																
Average Channel																

* FS parameter HV adjustment not available on some flow cytometers.

Figure 6: Target Intensity Standardization Protocol for Five Colors with Two Lasers Collected on a Cytomics FC 500 with RXP Software.

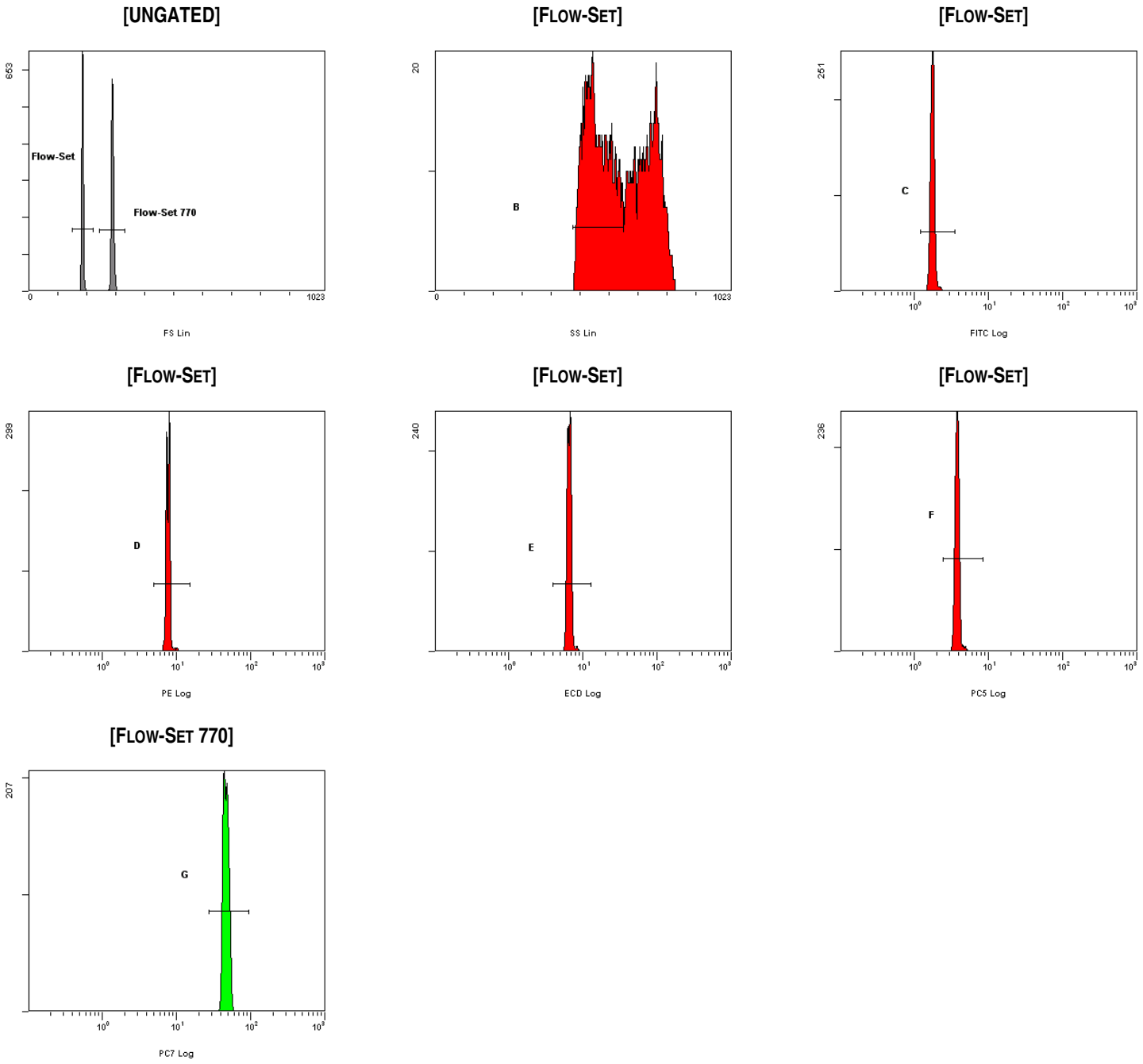

Figure 7: Example of a Table to Record Target Intensity Standardization Mode, High Voltage and Gain Settings when Establishing Instrument HV-Ranges.

ESTABLISHING HV/TOTAL GAIN RANGES

Application _____

FLOW-SET™ Fluorospheres Lot Number _____ Expiration Date _____

FLOW-SET™ 675 Fluorospheres Lot Number _____ Expiration Date _____

FLOW-SET™ 770 Fluorospheres Lot Number _____ Expiration Date _____

Mode Target Ranges:

FS _____ LOG FL1 _____ LOG FL3 _____

SS/LOG SS _____ LOG FL2 _____ LOG FL4 _____ LOG FL5 _____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4 HeNe ☐ 488 ☐		LOG FL5		
Run	Mode	HV*	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Tech/ Date
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
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15																	
16																	
17																	
18																	
19																	
20																	
Average HV/Gain																	
Average +2SD or +1%																	
Average -2SD or -1%																	

Serial No. _____

Lab. _____

Cytomics FC 500

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* FS parameter HV adjustment not available on some flow cytometers.

Figure 8: Example of a Levey-Jennings Chart

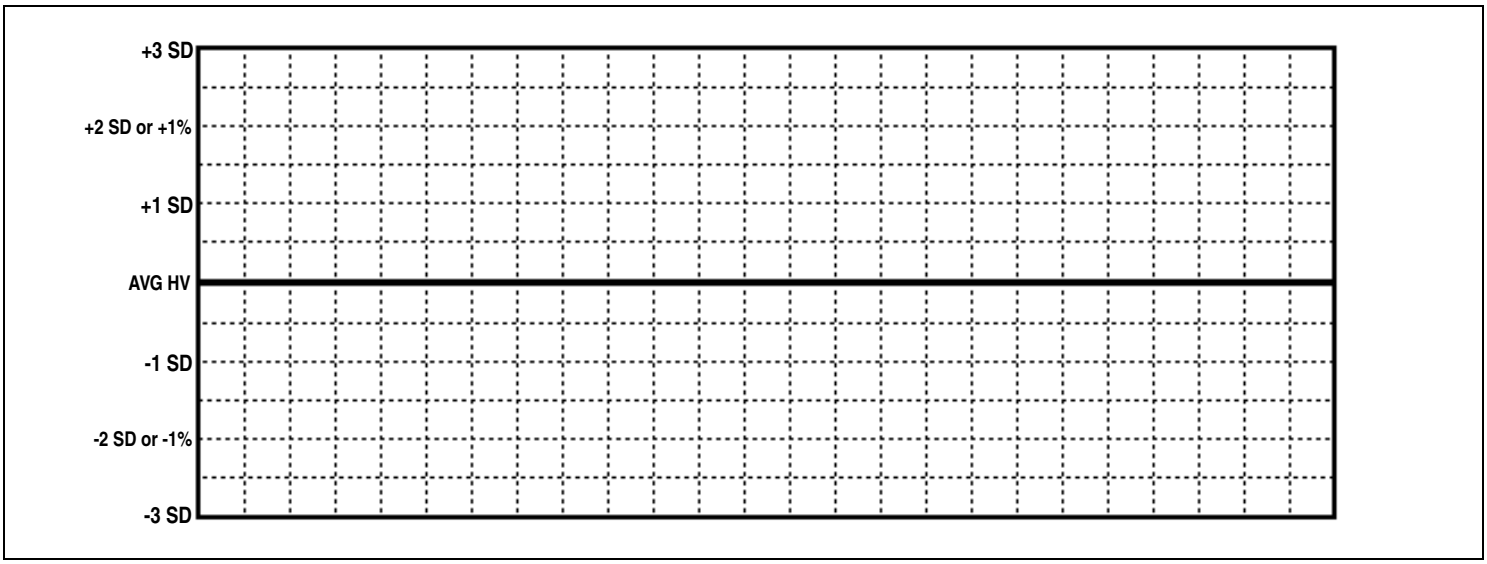

Figure 9: Example of a Daily Log to Record Detector Standardization Fluorospheres Mode, High Voltage, and Gain Settings.

DAILY LOG FOR INSTRUMENT STANDARDIZATION

Application _____

FLOW-SET™ Fluorospheres Lot Number _____ Expiration Date _____

FLOW-SET™ 675 Fluorospheres Lot Number _____ Expiration Date _____

FLOW-SET™ 770 Fluorospheres Lot Number _____ Expiration Date _____

Instrument HV/Total Gain Target Ranges:

FS _____ LOG FL1 _____ LOG FL3 _____

SS/LOG SS _____ LOG FL2 _____ LOG FL4 _____ LOG FL5 _____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4 HeNe ☐ 488 ☐		LOG FL5		
Run	Mode	HV*	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Tech/ Date
1																	
2																	
3																	
4																	
5																	
6																	
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30																	

Serial No. _____

Lab. _____

Cytomics FC 500

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*FS parameter HV adjustment not available on some flow cytometers.