



# **CyStain™ VitalCount reagents**

## **Quality Check Manual**

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## 1 Identification

The Quality Check procedure is applicable to:

### Reagent / Reagent kit

Name	CyStain™ VitalCount
Manufacturer	Sysmex Partec GmbH
REF	05-5029

### Device

Name	CyFlow™ Cube 6 V2m
Software	CyView™ 1.8 and above
Manufacturer	Sysmex Partec GmbH
REF	CY-S-3061R-V2m

## 2 Introduction

### 2.1 About this document

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This document will guide through the Quality Check (QC) procedure of the CyStain™ VitalCount reagent kits with a CyFlow™ Cube 6 V2m. Emphasis is placed on execution and troubleshooting.

Please refer to the CyFlow™ Cube 6 V2m manual for correct and safe use of the device.

For questions about this manual or the use of the device, please contact your local Sysmex representative.

### 2.2 About the procedure

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The Quality Check scripts simplify and automate the QC procedure of the CyFlow™ Cube 6 V2m for measurements with the CyStain™ VitalCount reagent kits. For analysis of the results, they are combined with associated FCS Express™ templates that are automatically launched during the QC procedure. It is necessary to apply specific Quality Check scripts and FCS Express™ templates corresponding to the used QC material LOT.

### 2.3 Installation and alteration

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The first installation must be performed by authorised service personnel.

No alterations should be made to the scripts and templates, unless expressly permitted by the manufacturer. After alteration of scripts or templates appropriate tests and trials must be performed to ensure safe use.

### 2.4 Sysmex representatives

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Find address and contact data of your local Sysmex representative at [www.sysmex-flowcytometry.com](http://www.sysmex-flowcytometry.com)

### 2.5 Authorised distributors

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To receive an overview of authorised distributors nearby, please contact your local Sysmex representative.

## 3 Overview

### 3.1 CyView™ script

The Quality Check procedure is integrated into a CyView™ configuration file (\*.cvc85) - called a CyView™ script. To access the QC procedure, the “QC”-Mode within the respective CyView™ script can be activated.

### 3.2 FCS Express™ template

FCS Express™ templates are used to automatically analyse the results of QC measurements. The templates can also be used to analyse data from QC measurements manually. The results can be exported.

### 3.3 Quality Check material

The QC procedure uses the following material(s). For further information please refer to the device manual.

#### NOTICE

##### Count Check Beads green specificity

This QC procedure was designed for the use with Count Check Beads green (REF 05-4011\_R). Similar products such as Count Check Beads green (REF 05-4011) are not suitable for use.

- Always use the recommended reagents.

Light source	Item	Content	REF
Blue Laser	Count Check Beads green	50 tests	05-4011_R

### 3.4 QC material LOT dependency

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The CyView™ script as well as the FCS Express™ templates are designed for a specific Count Check Beads green (05-4011\_R) LOT.

#### Check before QC procedures

LOT of used Count Check Beads green and revision of the script / templates are matching (see 4.2 Quality Check procedure).

“LOT N° mismatch” will be displayed in the FCS Express™ templates, if a template not matching the Count Check Beads green LOT was used.

The Quality Check is designed solely for QC material stated in chapter 3.3 Quality Check material. Similar products, such as Count Check Beads green (REF 05-4011) are not suitable.

#### Matching LOT and template

The LOT number (LOT N°) of the Count Check Beads green is composed of “NN00YYMMDD”. They are compatible with the script / template version, identical to the first 4 digits of the LOT N° “NN00”.

To check if the LOT matches, start the FCS Express™ template manually and type in the LOT N° of the Count Check beads green. If the LOT N° does not match “LOT N° mismatch” will be displayed.

Find the template at: *“C:\ProgramData\PartecGmbH\Cube\_18\templates\Quality Control”*

## 4 Operation

### 4.1 Software

CyView™ is used for control of the device, data acquisition, data analysis and storage. For more information, please refer to the CyFlow™ Cube 6 V2m manual.

FCS Express™ is used for reporting and analysis of data. The software can be accessed directly from within CyView™. Refer to FCS Express™ manuals and tutorials for detailed information about the software.

### 4.2 Quality Check procedure

#### 4.2.1 Setting up the Quality Check

##### Requirements

- Logged in to CyView™ with privileges of Main User level or above (Administrator)
- LOT and FCS Express™ template match, see chapter 3.4 QC material LOT dependency
- CyStain™ VitalCount configuration is active<sup>1</sup>.
- Priming has been performed
- Sample tube with 850 µl Count Check Beads green, see chapter 3.3 Quality Check material
- Sample tube with 1500 µl Sheath Fluid

##### Procedure

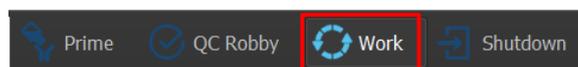


Figure 6: Selecting Work Mode

1. Select [Work Mode].
2. Switch Measure Mode Sample Source to “Sample Port”.
3. Connect the Sample tube with 850 µl Count Check Beads green.
4. Click [Start].
5. Adjust gain values in a way that the signals of the Count Check Beads green appear exactly in the center of the quality gate “QC (CCB)” within the main Plot.

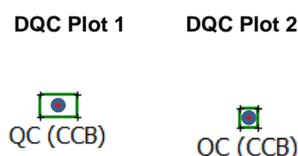


Figure 7: Adjust gain values

<sup>1</sup> E.g. VitalCount\_A\_AK01\_Robby.cvc85

## 4 Operation

6. Click [Stop].
7. Connect the Sample tube with 1500  $\mu$ l Sheath Fluid.



Figure 8: Intermediate Cleaning

8. Activate [Intermediate Cleaning].
9. Switch Measure Mode (Sample Source) back to “Autoloader”.
10. Switch Measure Mode (Sample Volume) to “51 – 100  $\mu$ l”.
11. Switch Measure Mode (Sample Mixing) to “High”.
12. Switch Measure Mode (Sample-to-Sample Cleaning) to “Intensive”.
13. Switch back to “Prime Mode” and save the configuration file as ...\_Robby.

### Result

The configuration file containing suitable gain values for all parameters can be saved on the device. It is recommended to maintain the default name for the configuration file. Continue by running a Quality Check.

### 4.2.2 Running a Quality Check

The QC procedure controls background, laser power, optical alignment, gate positioning and counting precision.

### Requirements

- Logged in to CyView™ with privileges of Main User or Administrator
- LOT and FCS Express™ template match, see chapter 3.4 QC material LOT dependency
- CyStain™ VitalCount configuration is active
- Priming has been performed
- 96-Well Plate is selected with QC material in the following order (e.g. A1)
  - e.g. A1 well with 200  $\mu$ l Count Check Beads green

## Procedure

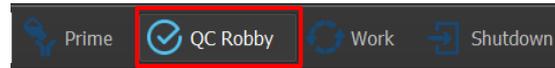


Figure 9: Selecting QC Robby Mode

1. Select [QC Robby Mode].
2. Press [Start] and follow the instructions.
3. The CyView™ Robby Autoloader AddOn will open. Assign a new Tray ID, e.g. “QCyyyymmdd”.

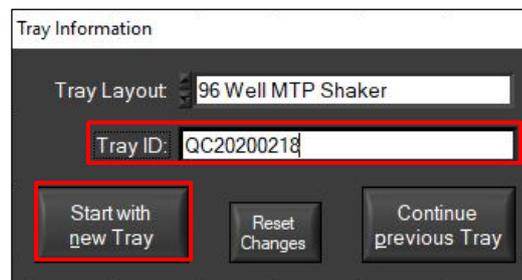


Figure 10: CyView AddOn

4. Click [Start with new Tray].
5. Select each with QC material and insert the Wellname “QC”. The correct order for the QC material is important when measuring the wells.
6. Click “Back to CyView”.

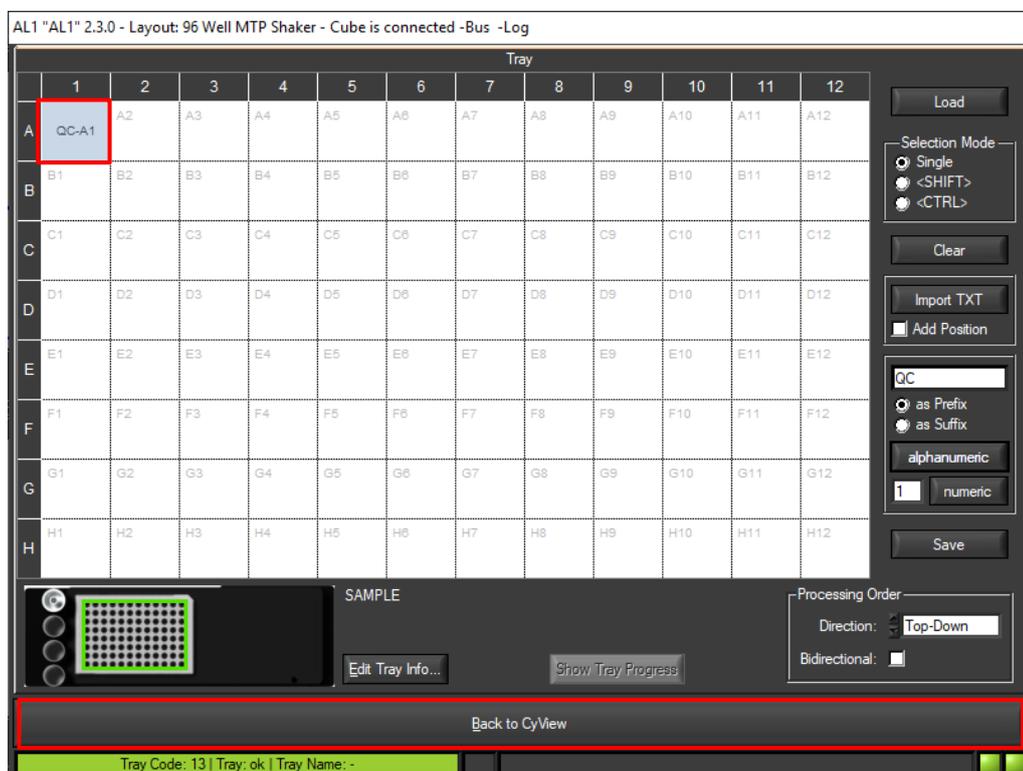


Figure 11: CyView TrayLayout

## 4 Operation

7. Press [Start] again to run QC Robby.



Figure 12: CyView Start Robby

8. Follow the instructions and Click [Continue] to start FCS Express.

### **NOTICE**

#### **Adjusting Device Settings during a Quality Check**

Signals should be visible in all dotplots. The predefined QC regions indicate the position where the signals of the beads are expected.

- If the beads signals are not within the predefined region, adjust the gain values , see chapter 4.2.1 Setting up the Quality Check.

### **Results**

The QC procedure results can be analysed in FCS Express™. Continue by analysing the Quality Check with FCS Express™.

### 4.2.3 Analysing the Quality Check with FCS Express™

FCS Express™ opens automatically, once the QC procedure has finished. The QC results will be analysed automatically by FCS Express™.

The first 4 digits of the LOT number need to be entered (e.g. “AK01”) in the first part of the LOT N° field. Make sure that the field contains no further characters, such as spaces.

The figure displays two side-by-side screenshots of the QC CyStain™ VitalCount interface. The left screenshot is labeled 'before Enter Quality Check material information' and the right is 'after Enter Quality Check material information'. Both screenshots show the same QC material: Sysmex Partec Count Check Beads green. The left screenshot shows the Lot N° field with 'Lot Num' and the Expiry Date field with 'YYY - MM'. The right screenshot shows the Lot N° field with 'AK01 19100' and the Expiry Date field with 'YYY - MM'. The Reference Concentration [per ml] is 'conc' on the left and '5000' on the right. The Report Summary shows 'Device Status : Lot N° mismatch' on the left and 'Device Status : VALID' on the right. The Tests performed section shows 'Background Check: PASS' and 'Laser Power: PASS' on both. The Information section shows 'CyView Operator: USER' and 'Sample ID: Count Check Beads Green.fcs' on both. The footer of both screenshots indicates the analysis was performed on 14.04.2021 at 11:08 by FSC-Express User S. Szustakiewicz.

Figure 13: Exemplary QC report (Page 1 / 2)  
(before Enter Quality Check material information) (after Enter Quality Check material information)

#	Description	#	Description
1	Input field Quality Check material information: LOT Number (example “AK01”)	5	Summary of performed tests Test: Pass or Fail
2	Input field Quality Check material information: Expiry Date	6	General information Device serial number, CyView Operator, Sample ID and Date
3	Input field Quality Check material information: Reference concentration	7	FCS Express™ template version
4	QC report summary: Device status: Valid or Invalid		

### Procedure

1. Switch to FCS Express™. Data have been transferred automatically to a predefined Layout.
2. Enter Quality Check material information for LOT N°, expiry date and reference concentration for Count Check Beads green.
3. The QC results are analysed automatically. Every test performed will “Pass” or “Fail” and a summary will be displayed.
4. To save a .pdf document of the results, switch to the Batch tab within FCS Express™ and click [Run]. Choose a suitable file folder and assign a data name. A PDF report will be generated and saved into the selected folder.

### Results

If all tests are passed, the device is qualified to perform a measurement with the reagent kit. If one or more tests fail, the device is not qualified to perform the measurement. Please refer to chapter 5 Troubleshooting.

### 4.3 Measurement procedure

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Please refer to the CyStain™ VitalCount kit manual as well as to the CyFlow™ Cube 6 V2m manual for instructions on performing measurements, saving acquired data as well as cleaning and shut down procedures.

## 5 Troubleshooting

### 5.1 Fault, cause and remedy

If the fault you are experiencing is not described or the remedy could not solve your problem, please contact your local Sysmex representative.

#### NOTICE

##### User Privileges

Options for troubleshooting depend on the user privileges. Controls for lasers, gain, flow rate and trigger parameter are only available at Main User level or above.

#### 5.1.1 Sheath Fluid, waste and fluids

Fault	Remedy
Sample flow is very slow or is not running	Check the flow rate settings.
	Check that the Waste bottle is closed properly and that the bottle is not cracked.
	Check all visible tubes and make sure that they are not pinched.
	Make sure that no air bubbles are trapped in the Inline Filter of the Sheath Fluid bottle.
	Perform priming procedure.
	Perform cleaning procedure.
	Shut down the device, including the Power switch on the back side of the device. Wait 3-5 minutes and restart the system.
Sample flow is very fast even with low flow rate	Check the speed settings.
	Change the Inline Filter in the Sheath Fluid bottle.
	Check if the visible Sheath Fluid tubing is pinched or blocked.
	Shut down the device, including the Power switch on the back of the device. Wait 3-5 minutes and re-start the system.

Fault	Remedy
The device emits an unusual / noisy sound after starting the measurement	Check that the Waste bottle is closed properly and that the bottle is not cracked.
	Check all visible tubing and make sure that they are not pinched.
	Check that liquid (Sheath Fluid and sample) is dropping into the Waste bottle after starting the measurement.

### 5.1.2 QC Procedure

Fault	Remedy
There is no data acquisition visible (peaks/dots in histogram/dot plot) during the RUN phase	Check within the software if the laser is switched on.
	Check if the right trigger parameter (FL1) has been selected.
	Check and adjust Gain values for all parameters
	Check threshold.
Unusual high background during measurement and /or failing background test within the Quality Check report	Make sure that no air bubbles are trapped in the Inline Filter of the Sheath Fluid bottle.
	Check threshold.
	Perform a priming procedure.
	Perform a cleaning procedure.
	Refill the Sheath Fluid. Sheath Fluid should be refilled every day.
	Clean the Sheath Fluid bottle and rinse it with Hypochlorite Solution.
	Renew the Inline Filter. The Inline Filter should be replaced every 3 months.
	Check the date of expiry of the QC Material.
<ol style="list-style-type: none"> <li>1. Remove the Inline Filter.</li> <li>2. Fill the Sheath Fluid bottle with Hypochlorite Solution and reconnect it.</li> <li>3. Switch to [Analyze All] and run three times 1200 µl Hypochlorite Solution at full speed.</li> <li>4. Refill the Sheath Fluid bottle with fresh Sheath Fluid and run three times 1200 µl Sheath fluid at full speed.</li> </ol>	
Failing Laser Power test within the Quality Check report	Check within the software if the laser is switched on.
	Check and adjust gain values for all parameters.
	Check the date of expiry of the QC material.
	Shut down the device, including the Power switch on the back side of the device. Wait 3-5 minutes and re-start the system.

Fault	Remedy
Unusual large CV values and/or failing Optical Alignment test within the Quality Check report	Perform a priming procedure.
	Perform a cleaning procedure.
	Check the date of expiry of the QC material.
	Shake the bottle of the QC material vigorously (e.g. by vortexing) and repeat the measurement.
	Check and adjust gain values for all parameters.
Count Check Beads green do not appear in the predefined regions of the configuration script and /or failing Gate Position test within the Quality Check report	Check within the software if you have loaded the correct configuration file for your measurement.
	Shake the bottle vigorously (e.g. by vortexing) and repeat the measurement.
	Check the date of expiry of the QC material.
	Perform a priming procedure.
	Perform a cleaning procedure.
Count Check Beads green measurement is not within $\pm 20\%$ range of the LOT-specific concentration stated on the bottle and /or failing Counting test within the Quality Check report	Shake the bottle vigorously (e.g. by vortexing) and repeat the measurement.
	Check the date of expiry of the Count Check Beads green.
	Perform a priming procedure.
	Perform a cleaning procedure.
	Refill the Sheath Fluid (ideally daily).
	Renew the Inline Filter. The Inline Filter should be replaced every 3 months.
“LOT N° mismatch” is displayed in the “Device Status” after entering the QC Material LOT number	Check and adjust Gain values for all parameters.
	Make sure to use a matching FCS Express™ template and Count Check Beads green LOT.  The LOT number of the Count Check Beads green is composed of “NN00YYMMDD”. They are compatible with script / template version identical to the first 4 digits of the LOT number “NN00”.