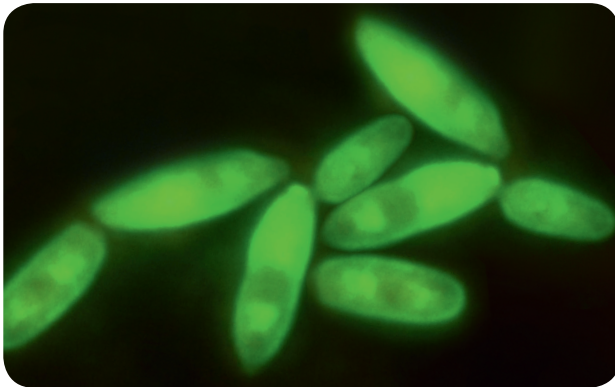




CyFlow™ BrettCount



Instructions for Use

Read this manual carefully before use.

MANUFACTURER



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By the use of this test kit living cells of *Brettanomyces bruxellensis* can be detected by flow cytometry specifically in wine and premature wine samples. The cells are fluorescently labelled and can be analysed in a flow cytometer with a 488 nm laser light source and forward and side scatter parameters as well as fluorescence detectors for green and red fluorescence.

The CyFlow™ BrettCount assay is optimized for Sysmex Partec CyFlow™ Cube 6 version 2M (Ref. No. CY-S-3060R_V2M). For this instrument an assay specific configuration script "BrettCount_A.cvc85" including a specific QC procedure and report template is available. For more information please contact your local Sysmex representative.

Notice: *The quality of the CyFlow™ BrettCount kits is monitored constantly and carefully. Each batch produced is routinely checked for functionality and specificity.*

STORAGE

Keep the kit and all its reagents stored at 2 – 8 °C.

Store the kit and all its reagents always upright and keep them protected from light.

SHELF-LIFE

Please refer to the expiry date stated on the kit.

PATENT INFORMATION:

The CyFlow™ BrettCount assay makes use of the patent protected *Flow VIT*® technology (vermicon AG).

MATERIALS

CONTENTS

Kit Reagents	Quantity
Solution W	1 x 17 mL
Solution P	2 x 25 mL
Solution H	1 x 1.7 mL
Solution S	1 x 0.4 mL
Solution G	1 x 0.4 mL
Solution D2F	1 x 1.7 mL
VIT F (Brett)	1 x 1.7 mL

REQUIRED EQUIPMENT

Sample Preparation	Specifications
Tubes, 2 mL, PP, sterile	IMPORTANT: round bottom
Vortex mixer	-
Centrifuge for 2 mL tubes	2000 – 4000 x g
Rack for 2 mL tubes	-
Heating block with 2 temperatures	For 2 mL tubes, 80 °C / 40 °C
Micro-pipettes & tips, sterile	1-20 µL, 10-100 µL, 100-1000 µL
Ethanol, absolute	> 99,8%, p.a.
Deionized water	-

Sample Analysis	Specification
Flow Cytometer	CyFlow™ Cube 6 version 2M (Ref No.: CY-S-3060R_V2M) Any flow cytometer with 488 nm excitation and FSC, SSC, FL green and FL red
Sample Tubes, 3.5 mL	04-2000
Caps for Sample Tubes 3.5 mL	04-2000-01
Sample Tube Rack	04-2000-02
Cleaning Solution	04-4009_R
Decontamination Solution	04-4010_R
Hypochlorite Solution	04-4012_R
Sheath Fluid	04-4007_R
Count Check Beads green	05-4011_R

SAMPLE PREPARATION PROCEDURE

Notice: Before starting the procedure, preheat one heating block to 80 °C (used in step D10). For step D12, cool down the heating block to 40 °C or use a second heating block (recommended).

SAMPLE PREPARATION

- D01.** Pipet 1 mL of wine sample into a 2 mL tube (round bottom).
- D02.** Pipet 300 µL of *Solution W* and 600 µL of *ethanol, absolute* (> 99,8%, p.a.) into the tube. Ensure a good blending of the solutions by manually turning the closed tube several times upside down.
- D03.** Centrifuge the sample (2000 – 4000 x g), 5 min at room temperature.
- D04.** Decant and discard the supernatant. Remove remaining supernatant at top tube edge carefully, e.g. with a clean laboratory tissue paper.
- D05.** Resuspend the pellet in the remaining liquid (vortex mixer, 5 sec).

SOLUTION PREPARATION

- D06.** Add 5 µL of *Solution G* (clear cap) into the tube from **D05**.
- D07.** Add 5 µL of *Solution S* (black cap) into the tube.
- D08.** Add 20 µL of *Solution H* (yellow cap) and 20 µL of *ethanol, absolute* (> 99,8%, p.a.) into the tube.
- D09.** Blend with vortex mixer for at least 5 sec.
- D10.** Open the tube and let it dry in a heating block at 80 °C for 60 min (before proceeding, check, that the sample is dry! If the sample is not dry, prolong drying incubation).

SAMPLE PREPARATION PROCEDURE

- D11.** Add 30 μ L of *VIT F Brett* (green cap) and resuspend the pellet (vortex mixer).
- D12.** Close the tube and incubate in a heating block at 40 °C for 90 min. After 15 min resuspend the sample.
- Notice:** *It is important to ensure that the pellet will be resuspended completely after 15 min (vortex mixer, at least 10 sec).*
- D13.** Add 30 μ L of *Solution D2F* (blue cap) and mix with vortex mixer for at least 5 sec.
- D14.** Close tube and incubate in a heating block at 40 °C for 15 min. Continue with Assay Measurement: CyFlow™ Cube 6 (see **M01** page 8) / Any Flow Cytometer (see **M01** page 12).

PREPARATION OF A MASTER MIX FOR STEPS D06 – D08

If more than one sample should be analysed at the same time, it is recommended to prepare a *Master Mix* for the solutions of step **D06 – D08**.

Master Mix – recipe per sample in a 2 mL tube:



- 5 μ l *Solution G*,
- 5 μ l *Solution S*,
- 20 μ l *Solution H*,
- 20 μ l *ethanol, absolute (> 99,8 %, p.a.)*
- Blend with vortex mixer for 5 sec.

Add 50 μ l of *Master Mix* to the tubes of step **D05**, mix with vortex mixer for 5 sec and continue with step **D10**. We do recommend preparing a *Master Mix* with 1 reaction in excess to ensure sufficient quantity also for the last reaction.


ASSAY MEASUREMENT

MEASUREMENT ON CYFLOW™ CUBE 6 (REF. NO. CY-S-3060R_V2M)

Notice: Set-up the CyFlow™ Cube 6 as described in “Assay set-up and quality control”.

- M01.** Add 940 µL of Solution P to the tube from step D14.
- M02.** Using the pipet (adjustable automatic pipet, 1000 µL) of step **M01**, mix the sample several times by up and down pipetting, then transfer the whole solution to a sample tube suited for the flow cytometer.
- M03.** Mix well using a vortex mixer, 5 sec.
- M04.** On the instrument switch to the [Work] stack  to activate the analysis layout (**Figure 1**, page 9).
- M05.** Connect the sample tube to the sample port.
- M06.** Click the [Start]  button to start the measurement.
- M07.** The measurement automatically stops at the end of analysis. 250 µL of wine sample will be counted (**Figure 2**, page 10).
- M08.** The data will be automatically saved (with date and time stamp) and transferred to the CyFlow™ BrettCount Report (**Figure 3**, page 11).
- M09.** The number of *Brettanomyces bruxellensis* cells per mL wine is indicated (content of PG3).

CLEANING BETWEEN MEASUREMENTS

- M10.** Fill a sample tube with 1.5 mL of deionized water.
- M11.** Connect the tube to the sample port and clean by clicking the [Clean]  button.

Subsequently the measurement of the next sample can be performed as described starting with step **M01**.

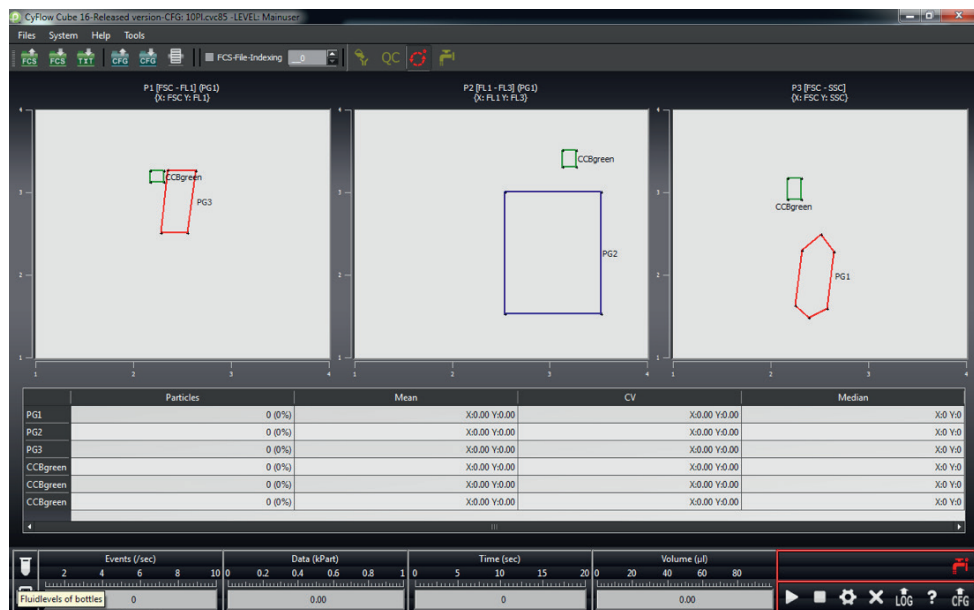


Figure 1: Configuration script BrettCount_A.cvc85

ASSAY MEASUREMENT

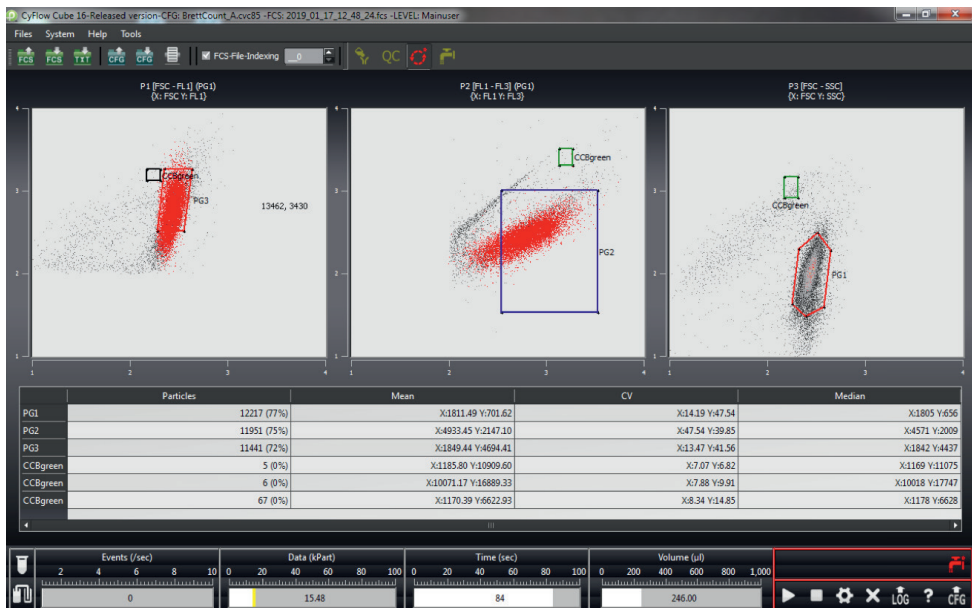


Figure 2: CyFlow™ BrettCount assay

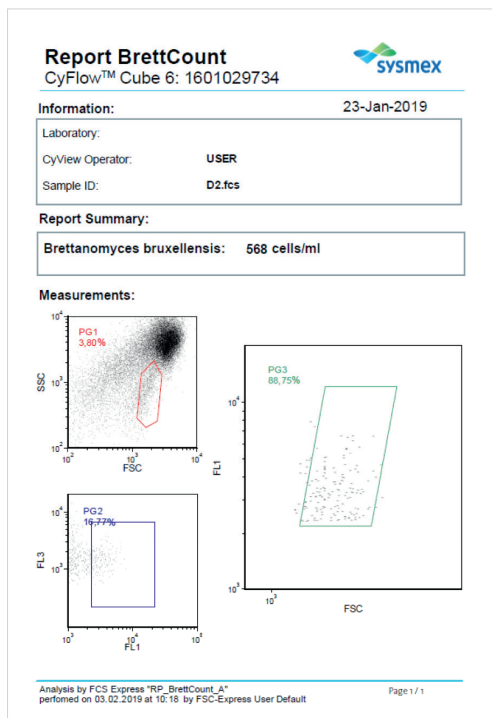


Figure 3: CyFlow™ BrettCount report. Cell count is based on the content of PG3.

ASSAY MEASUREMENT - ALTERNATIVE VERSION

ASSAY MEASUREMENT (ANY FLOW CYTOMETER)

- M01.** Pipet 940 μL of *Solution P* to the tube from step **D14**.
- M02.** Using the pipet (adjustable automatic pipet, 1000 μL) of step **M01** mix the sample several times by back and forth pipetting, then transfer the whole solution to a sample tube suited for the flow cytometer.
- M03.** Mix well using a vortex mixer.
- M04.** Analyze on a flow cytometer with suited optical configuration and setup with a minimum counting volume of 250 μL .

RECOMMENDED DATA ANALYSIS AND GATING STRATEGY:

- Create 3 dot plots, FSC vs SSC, FL (green) vs. FL (red) and FSC vs. FL (green).
- Prepare and run a positive control sample, e.g. *Brettanomyces bruxellensis* from culture and create 3 polygonal gating regions on the detected cell population (refer to Figure 2):
 - o PG1: FSC / SSC
 - o PG2: FL (green) / FI (red) gated on PG1
 - o PG3: FSC / FL (green) gated on PG2
- Count *Brettanomyces bruxellensis* cells in PG3.

For all wine samples use the pre-defined gating set and strategy.

ASSAY SET-UP AND QUALITY CONTROL

This chapter describes the Assay set-up and quality control of the instrument as well as the assay.



Start the instrument as described in the Operating Manual of the CyFlow™ Cube 6.

Prime the instrument according to the standard procedure.

The CyFlow™ BrettCount assay on CyFlow™ Cube 6 instruments with CyView™ software (version 1.6 or higher) operates with the dedicated configuration script “BrettCount_A.cvc85” (**Figure 1**, page 9) for sample measurement, the dedicated FCS Express template “RP_BrettCount_A.ley” (**Figure 3**, page 11) for data reporting and the QC report “QC_BrettCount_A.ley” (**Figure 6**, page 17) for assay setup and daily quality control.

ASSAY SET-UP AND QUALITY CONTROL

To adapt gain settings and verify proper instrument set-up for the CyFlow™ BrettCount assay follow the procedure described below:

1. Load the configuration script "BrettCount_A.cvc85".
2. Load the Quality Control stack by clicking on the [QC]  button within the top ribbon (**Figure 1**, page 9).
3. Press the [Start]  button.
4. Follow the instructions shown (**Figure 4**, page 15). You will be guided through the QC procedure.
5. The assay setup and quality control require Count Check Beads green. The beads signals should be positioned in each dot plot in the region "CCBgreen" (**Figure 5**, page 16).
6. In case the beads population is located outside of a "CCBgreen" region use the GAIN value of the correlated parameter to move the population into the "CCBgreen" region. Be aware that within one QC run the time to adjust the GAIN values is limited to 30 seconds.
7. At the end of the procedure QC data will be automatically transferred to the QC Report.
8. Enter bead concentration, lot number and expiry date of the used *Count Check Beads green*.
9. The result of the setup and QC is displayed on page 1 of the QC Report (**Figure 6**, page 17). Detailed information on the QC is displayed on page 2 of the report.

ASSAY SET-UP AND QUALITY CONTROL

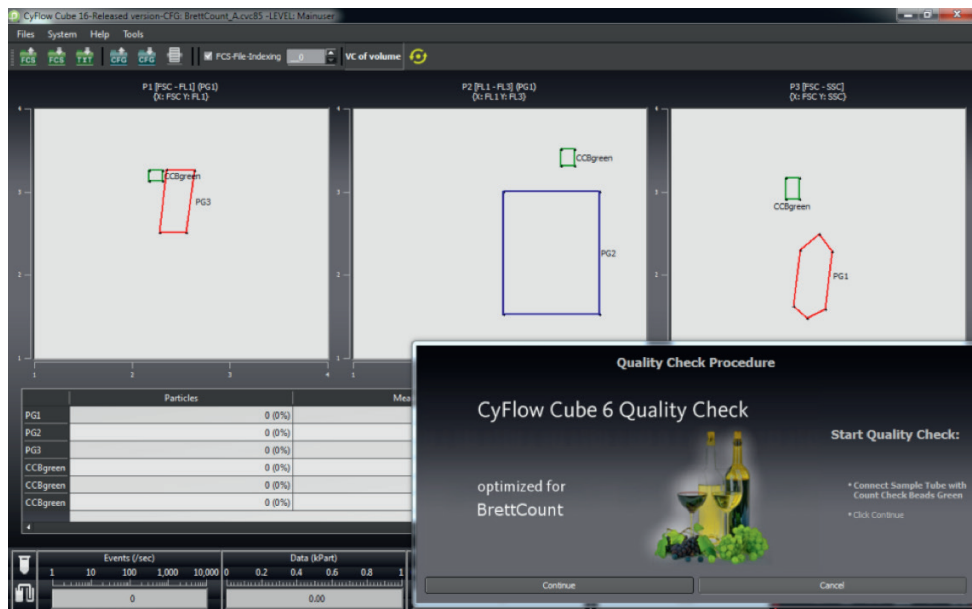


Figure 4: Quality Control procedure of Configuration script "BrettCount_A.cvc85"

ASSAY SET-UP AND QUALITY CONTROL

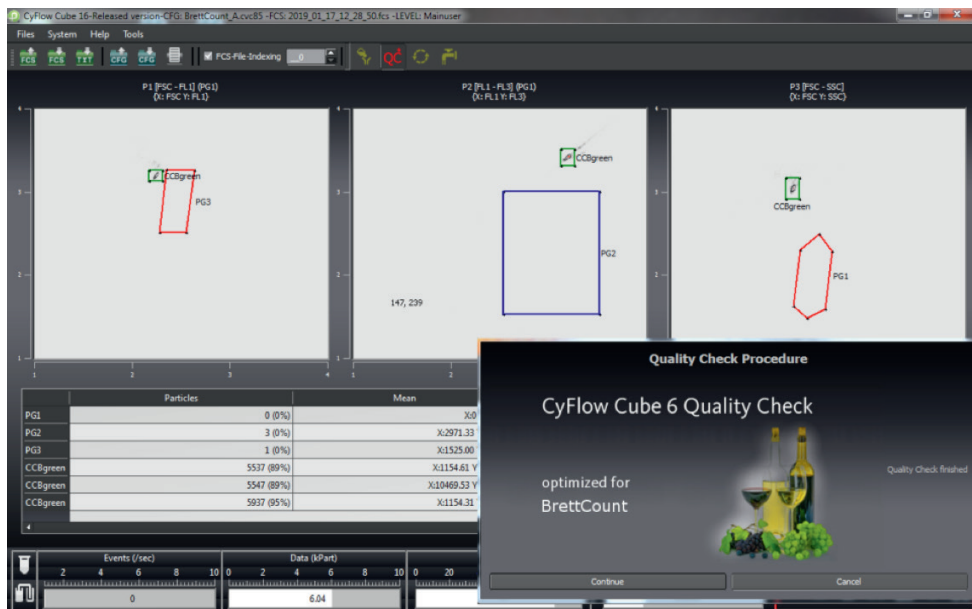


Figure 5: Finalized CyFlow™ Cube 6 Quality Check. Beads population in all 3 “CCBgreen” regions.

ASSAY SET-UP AND QUALITY CONTROL


QC Report BrettCount CyFlow™ Cube 6: 140610734	
	17-Jan-2019
QC Material: Sysmex Partec Count Check Beads green	
Lot N°:	SB180406
Expiry Date:	2019-06
Reference Concentration [per ml]:	24410
Report Summary:	
Device Status: VALID	
Tests performed:	
Background Check:	PASS
Laser Power:	PASS
Optical Alignment:	PASS
Gate Position:	PASS
Counting:	PASS
Information:	
Serial Number:	140610734
CyView Operator:	USER
Sample ID:	2019_01_17_12_28_50.fcs
Date of QC:	17-Jan-2019
Analysis by FCS Express "QC_BrettCount_A" performed on 17/01/2019 at 12:33 by FSC-Express User Default	
Page 1 / 2	

Figure 6: "QC_BrettCount_A" report page 1 with valid result.

HAZARDS AND PRECAUTIONS

CHEMICALS



GHS 05

Corrosion



GHS 08

Health hazard

Danger

Danger

HAZARD STATEMENT(S)

H318 Causes serious eye damage.

H350 May cause cancer.

H351 Suspect of causing cancer.

H360FD May damage fertility. May damage the unborn child.

H373 May cause damage to the blood through prolonged or repeated exposure.
Route of exposure: Inhalation.

PRECAUTION STATEMENT(S)

- P201** Obtain special instructions before use.
- P260** Do not breathe mist/vapours/spray.
- P280** Wear protective gloves/protective clothing/eye protection/face protection.
- P305+P351** IF IN EYES: Rinse cautiously with water for several minutes.
+P338 Remove contact lenses, if present and easy to do. Continue rinsing.
- P308+P313** IF exposed or concerned: Get medical advice/attention.
- P310** Immediately call a POISON CENTER/doctor.

Restricted to professional users.

For further information refer to the Safety Data Sheets.

Find Safety Data Sheets to our products at www.sysmex-partec.com.

DISPOSAL PROCEDURE

Notice: *Dispose of according all applicable regulations upon consultation of the local authorities and the disposer in a suitable and authorised disposal facility. Allocation of a waste code number, according to the European Waste Catalogue, should be carried out in agreement with the regional waste disposal company.*

SYMBOLS



Manufacturer



Reference number



Keep away from
sunlight



Caution



Use-by-date



Temperature limit



Batch code



|n| tests

TECHNICAL SUPPORT

For further questions or technical assistance, please contact your local Sysmex representative.

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