Read this package insert carefully before use

CyStain™ OenoCount **REF AN192657**

INTENDED USE

The CyStain™ OenoCount reagent kit is intended for the enumeration of viable microorganisms (bacteria and yeast) in an enriched and eluted wine sample as a final product control. CyStain™ OenoCount is intended to be used by trained personnel.

PRINCIPLE OF THE PROCEDURE

CyStain™ OenoCount is used to count viable microorganisms in samples of filtered wine by flow cytometric measurements. Preliminary sample preparation, e.g., by vacuum filtration and elution in CyStain™ Elution Buffer, is recommended to increase the concentration of microorganisms in the wine sample. The eluted sample is stained with two different fluorescent dyes included in the CyStain™ OenoCount kit.

CyStain™ Green is a membrane-permeable fluorescent dye staining all microorganisms in the wine sample and emitting green fluorescence. Both viable and non-viable cells will be stained.

CyStain™ Red is not membrane-permeable and stains cells that have a defective membrane and are categorised as non-viable cells.

STORAGE AND STABILITY

Unopened product

Store reagents at -25 °C to -18 °C, protected from light. Avoid repeated freeze-thaw cycles. Do not use after the expiration date stated on the label.

Product after first opening

The reagents can be used for 2 months after first opening. Return reagents to the freezer immediately after use. Do not use after the expiration date stated on the product label.

KIT COMPONENTS

Package contains the following reagents:

- 5 x 40 µL CyStain™ OenoCount Green, 1000X concentrate (in DMSO)
- 5 x 400 µL CyStain™ OenoCount Red, 100X concentrate
- 29 mL CyStain™ OenoCount Dilution Buffer

EVIDENCE OF DETERIORATION

Avoid contamination of reagents. In case of component deterioration or contamination seen as discoloration or turbidity of the reagent or if data obtained show any performance alteration, please contact the Technical Support of your local Sysmex representative.

HAZARD AND PRECAUTIONARY STATEMENTS

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet (available at http://www.sysmex-partec.com/services).

Always meet the national and international guidelines and regulatory standards for personal protective equipment (PPE).

ADDITIONAL REQUIRED EQUIPMENT

- Flow cytometer with a blue laser (488 nm) and detectors for forward scatter, side scatter, green and red fluorescence, e.g., Sysmex Partec CyFlow™ Cube 6 V2m
- Heating block / water bath set to 37 °C ± 0.5 °C
- Sample tubes compatible with the flow cytometer, e. g. sample tubes 3.5 mL (Ref. No. 04-2000, available from Sysmex Partec)
- 2 mL reaction tubes (Safe-Lock)

INSTRUCTIONS

NOTE: For instrument alignment and quality control, please refer to the instructions for use of your flow cytometer.

When using a Sysmex Partec CyFlow™ Cube 6 V2m, pre-defined measurement scripts including a quality check procedure to set up the voltage for all parameters are available. Please refer to the Quality Check Manual for the CyStain™ OenoCount reagents for detailed information.

Preparation of Staining Solutions

NOTE: Make sure all kit components are thawed completely. Please note that DMSO has a melting point of around 19 °C. Bring CyStain™ OenoCount Green to at least 19 °C before use.

1. Prepare a 1:100 working solution of CyStain™ OenoCount Green, 1000X concentrate, e.g., for 10 samples mix 10 µL CyStain™ OenoCount Green with 990 µL Dilution Buffer in a 2 mL reaction tube.
2. Vortex the reaction tube for 3 seconds.
3. Keep the working solution protected from light at 2-8 °C.

NOTE: The prepared working solution can be stored at 2-8 °C for up to 24 hours.

Sample Preparation

1. Add 900 µL of an enriched wine sample to a 2 mL reaction tube.
2. Add 100 µL CyStain™ OenoCount Green working solution to the wine sample.
3. Vortex the reaction tube for 3 seconds.
4. Incubate sample for 13 minutes at 37 °C ± 0.5 °C in a heating block or water bath, protected from light.
5. Add 10 µL of CyStain™ OenoCount Red, 100X concentrate.

6. Vortex the reaction tube for 3 seconds.
7. Pipette 850 µL of sample into a sample tube for flow cytometry.
8. Analyse sample with a flow cytometer.

Recommended data analysis and gating strategy

NOTE: When using a Sysmex Partec CyFlow™ Cube 6 V2m, pre-defined measurement scripts and analysis templates are available.

If you are using an instrument from another manufacturer, use the following instructions as a guide for setting up your instrument for analysis:

- Create 2 dot plots with logarithmic scale: “P1”: FL1 (green fluorescence) vs. FL3 (red fluorescence), “P2”: FL1 (green fluorescence) vs. FSC.
- Select FL1 (green fluorescence) as trigger parameter.
- Adjust gain values for FSC, FL1 and FL3.
- Run the wine sample and create 3 polygonal gating regions on bacterial and yeast cell populations:
  “Viable MO”: FL1 vs. FL3 (viable microorganisms),
  “Small MO”: FL1 vs. FSC (small microorganisms, mainly bacteria),
  “Large MO”: FL1 vs. FSC (large microorganisms, mainly yeast).
- Apply the gate “Viable MO” to FL1 vs. FSC.
- Count viable microorganisms in “Viable MO.” (Fig. 1), count viable small microorganisms in “Small MO” (Fig. 2) and count viable large microorganisms in “Large MO” (Fig. 2).

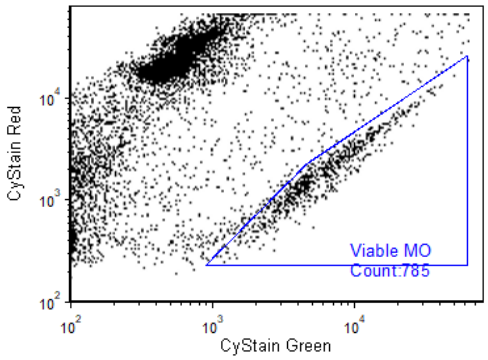

Figure 1: Analysis of a red wine sample: CyStain™ Green plotted vs. CyStain™ Red. Viable microorganisms are located in the blue "Viable MO" region

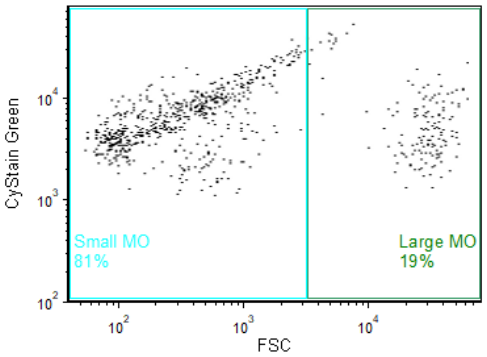

Figure 2: Analysis of a wine sample spiked with various microorganisms: Cell size (FSC) vs. CyStain™ Green separating small and large MO

DISPOSAL PROCEDURE

Disposal procedure should meet requirements of applicable local regulations.

MANUFACTURER

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SYMBOLS

REF	Reference number		Consult instructions for use
LOT	Batch code		Manufacturer
	Use-by date		Temperature limit
Σ	Contains sufficient for <n> tests		Keep away from sunlight