Read this package insert carefully before use

CyStain™ BacCount Total **REF 05-5008**

The CyStain™ BacCount Total reagent kit is intended for the unspecific enumeration of all bacteria (Total Cell Count) in water samples. CyStain™ BacCount Total is intended to be used by trained personnel.

KIT COMPONENTS

Packing contains the following reagents:

- 5 Aliquots x 40 µL CyStain™ Green (1000X concentrated in DMSO)
- 29 mL CyStain™ Dilution Buffer

PRINCIPLE OF EXAMINATION METHOD

CyStain™ Green is a membrane permeable dye that unspecifically stains all bacteria in water samples, emitting green fluorescence. Both live and dead cells will be stained.

ADDITIONAL REQUIRED EQUIPMENT

- 2 mL reaction tubes (Safe-Lock)
- Sample tubes for flow cytometry, e g. Sample tubes 3.5 mL (Ref. No. 04-2000) for Sysmex Partec instruments
- Heating block/water bath set to 37 °C ± 0.5 °C
- CellTrics™ filters 50 µm (Ref. No. 04-0042-2317)
- A flow cytometer with a blue laser (488 nm) and detectors for forward scatter, side scatter, green fluorescence and red fluorescence. The Sysmex Partec CyFlow™ Cube 6 V2m is recommended.

INSTRUCTIONS

Preparation of Staining Solution

NOTE: Make sure that all kit components have thawed.

1. Prepare a 10X working solution by diluting the 1000X stock solution of CyStain™ Green 1:100 with CyStain™ Dilution Buffer.
2. Mix working solution with a vortex mixer for 3 seconds.
3. Keep working solution protected from light.

Sample Preparation

NOTE: Samples with larger particles as impurities have to be filtered prior to staining and measurement. As a filter, a CellTrics™ filter 50 µm is recommended.

4. Mix 100 µL CyStain™ Green working solution with 900 µL water sample in a 2 mL reaction tube.
5. Mix sample with a vortex mixer for 3 seconds.
6. Incubate sample for 13 minutes at 37 °C ± 0.5 °C, protected from light in a heating block or water bath.
7. Mix sample with a vortex mixer for 3 seconds.
8. Pipette 850 µL of the sample into a sample tube for flow cytometry.
9. Analyse sample with a flow cytometer.

Recommended data analysis and gating strategy

NOTE: When using a Sysmex Partec CyFlow Cube 6 V2m, use the pre-defined gates to set up the voltage for all parameters using 1 mL of diluted Calibration Beads 0.5 µm (< 2x10^5 beads per mL; Sysmex Partec, Ref. No. 05-4005) and Count Check Beads Green (Sysmex Partec Ref. No. 05-4011_R).

- Create 2 histograms with a logarithmic scale: "H1" SSC, "H2" FL1 (green fluorescence)
- Create 2 dot plots with logarithmic scale: "P1" FL1 (green fluorescence) vs. FL3 (red fluorescence), "P2" FL1 (green fluorescence) vs. SSC
- Select FL1 (green fluorescence) as trigger parameter.
- Adjust gain values for SSC, FL1 and FL3
- Run a water sample (e. g. "evian" mineral water) and create 3 polygonal gating regions on bacterial cell populations: "PG1" FL1 vs. FL3 (total bacteria), "PG2" FL1 vs. SSC (LNA bacteria), "PG3" FL1 vs. SSC (HNA bacteria)
- Count total bacterial cells in "PG1" (Fig. 1), Count total LNA bacterial cells in "PG2" (Fig. 2), Count total HNA bacterial cells in "PG3" (Fig. 2)

NOTE: Pre-defined measurement scripts with appropriate instrument settings are available for the CyFlow™ Cube 6 V2m. Please contact your local Sysmex representative for further information.

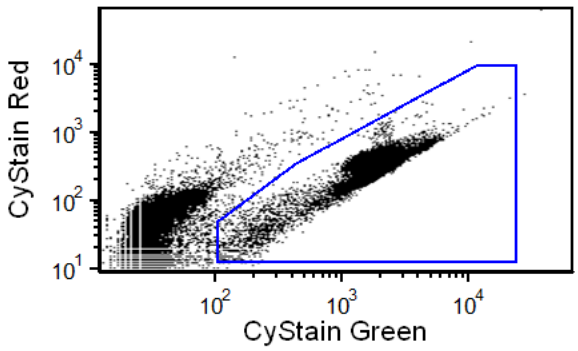

Fig. 1: CyStain™ Green vs. CyStain™ Red with gate PG1

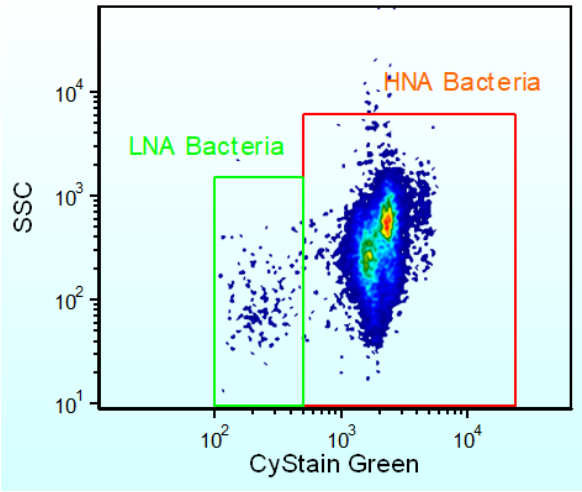

Fig. 2: "PG2" LNA bacteria and "PG3" HNA bacteria

STORAGE AND STABILITY

Storage

Store at -25 °C to -18 °C protected from light, avoid repeated freeze-thaw cycles.

Shelf life

Please refer to the expiration date on the product label.

The reagents are stable for 2 months after opening.

HAZARD AND PRECAUTIONARY STATEMENTS

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

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

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

PERSONAL PROTECTIVE EQUIPMENT

When using the reagent(s) make sure to wear suitable personal protective equipment. For additional information please consult Safety Data Sheet(s).

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		Temperature limit
Σ	Content sufficient for <n> tests		Keep away from sunlight