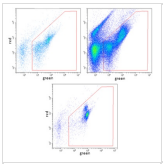

Nov 30, 2020

Determining the total cell concentration (TCC) in drinking water with flow cytometry

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol describes how to stain cells in (drinking) water samples with SYBR Green I for the purpose of total cell concentration measurements and flow cytometric fingerprinting. The protocol is based on the studies of Prest et al. (2013) and Van Nevel et al. (2013). The protocol is applicable for different types of water samples, and based on the flow cytometric equipment, the volumes of samples and reagents can simply be adjusted.

Prest EI, Hammes F, Kötzsch S, van Loosdrecht MC, Vrouwenvelder JS (2013). Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method.. Water research.
https://doi.org/10.1016/j.watres.2013.07.051

Van Nevel S, Koetzsch S, Weilenmann HU, Boon N, Hammes F (2013). Routine bacterial analysis with automated flow cytometry.. Journal of microbiological methods.
https://doi.org/10.1016/j.mimet.2013.05.007

PROTOCOL CITATION

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KEYWORDS

Bacteria, Drinking Water, Water Quality, Cell Concentration, Flow Cytometry, Staining

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MATERIALS TEXT

- Flow cytometer equipped with blue laser (488 nm) and detectors for green (520 nm) and red (620+) fluorescence
- Heating block or incubator (**37 °C**)
- SYBR Green I® working solution: dilute SYBR Green I® stock solution 1:100 in sterile, filtered TRIS Buffer (10 mM, pH 8)
- Suitable sample tubes or multi-well plates for the flow cytometer used
- Filtered (**0.1 µm**) bottled water (e.g., EVIAN®, France) or buffer (e.g., TRIS Buffer; 10 mM, pH 8) for dilution if required
- Minimum **1 mL** of water sample

SAFETY WARNINGS

SYBR Green I®
Skin protection: Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. (Information: https://www.thermofisher.com/order/catalog/product/S7585#/S7585)

ABSTRACT

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BEFORE STARTING

Make sure that your flow cytometer is properly cleaned and calibrated and the correct instrument settings are activated.

1 Dilute the water sample with 0.1 µm filtered water (filtered bottled water, original sample or buffer) if a concentration of more than 1 x 10^6 cells/mL is expected. Multiple samples can be prepared and processed simultaneously.

2 Transfer **500 µl** of the water sample into a labelled 3.5 mL sample tube. Or the corresponding volume when using other sample tubes.

3 Add **5 µl** of SYBR Green I® working solution (1:100 dilution of stock solution) to the tube and vortex briefly. The final SYBR Green I® dilution in the sample is therefore 1:10'000.

SYBR Green I® has DNA-binding properties. It is recomended to wear gloves for this step.
(see additional infos under "Warnings")

4 Incubate for a minimum of **00:10:00 Min.** at **37 °C** in the dark. Longer incubation is not problematic, but should be limited to a maximum of **30 min**. 10m

5 Vortex briefly and measure a volume of at least **50 µl** on a cleaned and calibrated flow cytometer with appropriate settings, using green fluorescence as the main trigger/threshold parameter.

6 Create a density plot of green fluorescence (520 nm) vs. red fluorescence (620+ nm)

7 Distinguish between microbial cells and background noise with the gating feature of the flow cytometry software.