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Bacterial SYBR-Cell Staining and Counting with the Attune Flow Cytometer

Forked from a deleted protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.3byl4w3nzvo5/v1>

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Protocol status: Working

We use this protocol and it's working



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Protocol Integer ID: 117417

Keywords: bacterial sybr, counting with the attune flow cytometer, cell staining, sybr cell, attune flow cytometer, cell

Abstract

SYBR Cell staining in MICO lab

Prep

- 1 Clean culture hood with 70% ethanol and use UV light to sterilize the hood (about 15 minutes)
- 2 Pre-heat mini-incubator to 37°C.
- 3 Take foil-wrapped diluted SYBR stock out from fridge door and allow it to come to room temperature (about 5-10 minutes), while keeping it protected from light.
- 4 After warmed, vortex the SYBR stock and make sure it is well mixed
- 5 Take out samples for the day (in fridge, labeled with station, depth, date, and "BACT") and gently vortex each sample.

Note: cruise 3 has BACT 1 and 2 so that we have enough volume for the imaging flow cyto as well

Staining

- 6 put a fresh 96-well plate, mixed samples, and mixed SYBR stock into the cleaned culture hood
- 7 Add 1 (one) μ l of SYBR stock to each well you intend to use in the assay. Make sure to add the 1 μ l to the bottom center of the well so that you can see it.

- do not use the furthest column to the right
- 8 add 99 (ninety-nine) μ l of water to well 1A - this will be a blank
- 9 add 99 (ninety-nine) μ l of sample to each of the other wells and mix 5-10x so that SYBR and samples are mixed.



	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											Do
B												not
C												use
D												
E												
F												
G												
H												

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This can be printed and used to write in sample names

10 Record sample-well locations

Either print template, use the postits, or record in protocols (makes sure you save run):

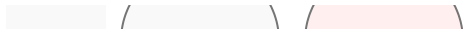
	1	2	3	4	5
A	Blank				
B					
C					
D					
E					
F					
G					
H					
	6	7	8	9	10
A					

B					
C					
D					
E					
F					
G					
H					
	11	12			
A					
B					
C					



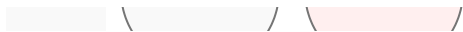
Incubate

- 11 put sample plate into the mini-incubator and incubate at 37°C for 10 minutes



Edit Attune Template

- 12 Open proper experiment from template and edit sample names to match actual sample names



Run Experiment on Attune

- 13 use bacterial template

