Cell Harvesting Protocol

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[1]In-house protocol

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

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ABSTRACT

A general procedure for harvesting bacterial cells for flow cytometry or fluorescence microscopy
Write up by S. Shore 11/17/2020 based on B. Bogati communications, proofread by Bikash Bogati

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ABSTRACT

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Procedure

1 Dilute the overnight culture to OD_{600} 0.01 and grow cells in an appropriate medium to the desired time point/OD_{600} in at least 10mL of liquid culture.

2 Harvest cells directly into pre-labeled falcon tubes (if running flow same-day) or Eppendorf tubes. Once cells have been harvested, minimize exposure to direct light if looking for fluorescence.
1. For *E. coli* from overnight LB, at least 25uL
2. For *E. coli* from exponential phase (OD~0.3) LB, at least 50uL
3. For *E. coli* from the overnight or exponential phase in minimal media, at least 500uL

3 For membrane depolarization assay:
1. Wash the cells twice with 1x PBS and resuspend in 1 ml PBS
2. Add $DiBAC_4$-3 reagent- final concentration 10mg/mL (4ml of 2.5 mg/mL working stock in 1 mL)
3. Mix and incubate in dark for 20 minutes. Wash twice in PBS.
4. Fix using paraformaldehyde and glutaraldehyde (see below)

4 Optional: Fix with desired fixation method in the chemical fume hood
1. Required if using the LSR-II in Mossman. Special permission needed to run live-cells through a flow cytometer.
2. Potential fixation methods:
   - 0.5% paraformaldehyde for 10 min in a cold room in dark (T. Hancock)
   - 3% glutaraldehyde for 1hr at room temp in dark (R. Johnston)
   - paraformaldehyde to final concentration 2.8% (vol/vol) and glutaraldehyde to 0.04% (vol/vol) for 15 min at room temp in dark (Dr. J.Mannik)

5 Wash cells 3 times
1. Centrifuge at 4 degrees until pellets form (tabletop for 3 min, if in falcon tubes, 10 min in a large centrifuge)
2. Decant supernatant in an appropriate chemical waste container in the hood
3. Flood sample with PBS and vortex
4. Repeat steps 1-3 2 additional times

6 Decant liquid and add PBS to the desired volume.
1. For flow and E. coli, 1 mL for minimal media samples, 0.5 mL for LB samples if the followed recommendation in step 2.
2. If mounting cells on slides for microscopy, do not add PBS during the last round. Aspirate remaining liquid out instead.

7 If applicable, store cells in the fridge (4 degrees).
1. If using the J.Mannik fixation method, cells can be stored up to a month in the dark.