Fixation of fluorescent E.coli by Mannik

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[1]In-house protocol

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1 Works for me

This protocol is published without a DOI.

Eadewunm

ABSTRACT

Per Jaana Mannik Communication, June 1st 2020:
For the fixation of FtsZ-mNG cels, we have been using a mix of paraformaldehyde (PFA) and glutaraldehyde (see mu protocol below). In the case of FtsZ, we will lose a specific Z-ring-like structure using only PFA; the addition of glutaraldehyde helps to preserve it.

If your protein of interest is not sensitive to stress response, the response fixation with only PFA should work well. fixation with PFA should maintain fluorescence signal as it is.

PROTOCOL CITATION

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Fixation of FtsZ-mNG E.coli cells

1 Fix cells directly in growth medium by addition of paraformaldehyde (PFA) to final concentration 2.8% (vol/vol) and glutaraldehyde (GH) to 0.04% (vol/vol) for 15 minutes at RT (work with PFA and GH under the chemical hood!)

2 Transfer the cells to epis and spindown at 6000 rpm for 1:30 minutes

3 Remove supernatant (under the chemical hood; collect supernatant to PFA/GH waste bottle)

4 Wash 2x with PBS: add 1ml of 1xPBS and resuspend the pellet, spin down at 6000 rpm for 1:30 minutes, remove super. Repeat the step. (RT or 4^{O}C). The cells can be stored up to a month at 4^{O}C. Keep the cells in the dark