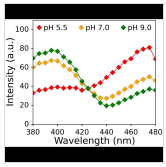

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Cytoplasmic pH measurements with pHluorin

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bhi7j4hn

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SUBMIT TO PLOS ONE

ABSTRACT

Single-cell *in vivo* cytoplasmic pH measurments with genetically encoded pHluorin sensor

EXTERNAL LINK

https://www.nature.com/articles/s41598-019-55798-0

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Wang, Y., Krasnopeeva, E., Lin, S. et al. Comparison of Escherichia coli surface attachment methods for single-cell microscopy. Sci Rep 9, 19418 (2019). https://doi.org/10.1038/s41598-019-55798-0

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

null, single cell measurements, pH measurements, epifluorescence measurements

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MATERIALS TEXT

MATERIALS

Potassium benzoate ReagentPlus® 99% **Sigma Aldrich Catalog #290009-250G**

Methylamine hydrochloride **Sigma Aldrich Catalog #M0505-100G**

SAFETY WARNINGS

pH adjustment requires work with acid or base solutions. Proper PPE should be worn and chemical waste should be appropriately disposed of.

Sample preparation

1 Grow cells to the desired OD according to a chosen growth protocol.

2 Prepare a tunnel-slide/flow cell and attach cells to the coverslip with the preferred surface attachment method (Wang et al. 2019)

It is preferable to use a non-fluorescent medium for imaging. If needed you can clean your coverslips with KOH or by baking them.

Cells imaging

3 Cell imaging is done by custom-made microscope with Nikon CFI Plan Apochromat Lambda 100x objective (NA 1.45) and iXon Ultra 897 EMCCD camera.
Narrow spectrum 395 nm LED is used for the excitation at 395 nm and Neutral white LED with ET470/40x filter for 475 nm excitation. Emission is collected at 520 nm using ET525/40m filter.
Imaging settings vary depending on the experiment.

ET520/40m
Narrow band emission filter
Chroma Technology **ET520/40m**

ET470/40x
Narrow band filter
Chroma Technology **ET470/40x**

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iXon Ultra 897 EMCCD
EMCCD camera

Andor **iXon Ultra 897 EMCCD**

CFI Plan Apochromat Lambda 100X Oil
Objective

Nikon **CFI Plan Apochromat Lambda 100X Oil**

pH sensor calibration

4 Perform the pH sensor calibration using the same conditions and imaging parameters as the main experiment. Supplement experimental medium with pH collapsing agent PBMH (40 mM potassium benzoate and 40 mM methylamine hydrochloride) and adjust its pH to the range of values between 5.5 and 9.

5 Flush the experimental medium of known pH into a sample chamber (when using a tunnel slide we wick it into the tunnel with a pipette at one entrance of the channel and a small tissue on the other), incubate for 15~min. Take images of 5-10 different fields of view containing at least 100 cells in total with the same imaging parameters as the main experiment.

6 Analyse images recovering emission intensities for 395 and 475 nm excitation channels. We use custom-written Python script to calculate mean intensities of the single cells' cytoplasm fluorescence.

7 Plot the calibration curve as a ratio of emission intensities for excitation at 395 nm and 475 nm against pH.
Fit with the sigmoid function $R_{395/475}=(a_1 e^{k(pH-pH0)}+a_2)/(e^{k(pH-pH0)}+1)$, where a_1, a_2, k and pH_0 are free fitting parameters.
Use the calibration curve to recover pH from intensity ratio data.

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