MAR 02, 2021

Cytoplasmic pH measurements with pHluorin

In 1 collection

Ekaterina
Krasnopeeva¹,

Teuta
Pilizota¹

¹University of Edinburgh

Ekaterina Krasnopeeva: Currently at IST Austria

Teuta Pilizota

Ekaterina Krasnopeeva

ABSTRACT

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

MATERIALS

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.

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bhi7j4hn

External link:

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

Protocol Citation: Ekaterina Krasnopeeva, Teuta Pilizota 2021. Cytoplasmic pH measurements with pHluorin . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bhi7j4hn>

MANUSCRIPT CITATION:

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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status:

Working
We use this protocol and it's working

Created: Jun 15, 2020

Last Modified: Mar 02, 2021

PROTOCOL integer ID:
38207

Keywords: single cell
measurements, pH
measurements,
epifluorescence
measurements

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](#))

Note

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.

Equipment

ET520/40m

NAME

Narrow band emission filter

TYPE

Chroma Technology

BRAND

ET520/40m

SKU

<https://www.chroma.com/products/parts/et520-40m>

LINK

Equipment

ET470/40x

NAME

Narrow band filter

TYPE

Chroma Technology

BRAND

ET470/40x

SKU

<https://www.chroma.com/products/parts/et470-40x>

LINK

Equipment

iXon Ultra 897 EMCCD

NAME

EMCCD camera

TYPE

Andor

BRAND

iXon Ultra 897 EMCCD

SKU

<https://andor.oxinst.com/products/ixon-emccd-camera-series/ixon-ultra-897>

LINK

Equipment

CFI Plan Apochromat Lambda 100X Oil

NAME

Objective

TYPE

Nikon

BRAND

CFI Plan Apochromat Lambda 100X Oil

SKU

https://www.nikon.com/products/microscope-solutions/lineup/objectives/cfi_plan_apochromat_lambda/index.htm

LINK

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 $R_{395/475} = (a_1 e^{k(pH-pH_0)} + a_2) / (e^{k(pH-pH_0)} + 1)$, where a_1 , a_2 , k and pH_0 are free fitting parameters.
Use the calibration curve to recover pH from intensity ratio data.