Jan 11, 2026

Measurement of Lysosomal pH Using LysoSensor Yellow/Blue DND-160

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7nrx1lwz/v1

Ali Ghoochani[1,2,3,4], Monther Abu-Remaileh[1,2,3,4]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA;
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA;
[3]The Institute for Chemistry, Engineering and Medicine for Human Health (Sarafan ChEM-H), Stanford University, Stanford, CA 94305, USA;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

asap

Ali Ghoochani
Stanford University

DOI: https://dx.doi.org/10.17504/protocols.io.q26g7nrx1lwz/v1

Protocol Citation: Ali Ghoochani, Monther Abu-Remaileh 2026. Measurement of Lysosomal pH Using LysoSensor Yellow/Blue DND-160. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7nrx1lwz/v1

Protocol status: Working
We use this protocol and it's working

Created: July 08, 2025

Last Modified: January 11, 2026

Protocol Integer ID: 222007

Keywords: measurement of lysosomal ph, quantitative lysosomal ph value, lysosomal ph, ratiometric dye lysosensor yellow, using lysosensor yellow, lysosensor yellow, fluorescence measurement, ph calibration curve, fluorescence, dye incubation

Abstract

This protocol describes the measurement of lysosomal pH using the ratiometric dye LysoSensor Yellow/Blue DND-160. The method includes dye incubation, fluorescence measurement, and the use of a pH calibration curve to derive quantitative lysosomal pH values.

Materials

- LysoSensor Yellow/Blue DND-160 (Thermo Fisher Scientific)
- 1x DPBS
- MES calibration buffers (pH 3.5–7.0)
- eBioscience Monensin Solution (1000X) (Thermo Fisher Scientific, Cat# 00-4505-51)
- Nigericin sodium salt (Sigma, Cat# SML1779)
- Microplate reader capable of dual excitation/emission readings

Materials

1

LysoSensor Yellow/Blue DND-160 (Thermo Fisher Scientific)
1X DPBS
MES calibration buffers (pH 3.5–7.0)
eBioscience Monensin Solution (1000X) (Thermo Fisher Scientific, Cat# 00-4505-51)
Nigericin sodium salt (Sigma, Cat# SML1779)
Microplate reader capable of dual excitation/emission readings

MES Calibration Buffer Composition:

2

- 20 mM MES
- 20 mM NaCl
- 110 mM KCl
- 1x monensin
- 20 µM nigericin

Procedure:

20m

3 Culture SH-SY5Y cells in complete media. Once cells become 90% confluence, wash cells with 1XDPBS and add serum-depleted DMEM for 72 hours.

Note

This is a general protocol for measuring lysosomal pH. It can be applied to a variety of cell types, with or without serum stravation depending on the specific experimental conditions.

4 Wash cells once or twice with 1x DPBS.

5 Incubate cells (experimental cells and cells for generating pH calibration curve) with 5 µM LysoSensor Yellow/Blue DND-160 in DMEM for 00:10:00 at 37 °C

10m

6 Optional: Wash cells once with 1x DPBS.

7 Generate a calibration curve by equilibrating LysoSensor-loaded cells in MES calibration buffers with pH values ranging from 3.5 to 7.0.

8 Incubate cells in each buffer for 00:10:00 at 37 °C before measuring fluorescence. 10m

9 Measure fluorescence using a microplate reader at the following settings:
- Excitation at 340 nm → Emission at 440 nm
- Excitation at 380 nm → Emission at 540 nm

10 Calculate the emission ratio (440/540 nm), which correlates with lysosomal pH.

11 Use the calibration curve to interpolate lysosomal pH from measured emission ratios.