

Intracellular pH Calibration Buffer Kit

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Revision 1.0

Detailed protocol is available online at www.lifetechnologies.com/manuals.

WARNING! For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Chemical	CAS	Warning
DMSO	67-68-5	Components of the product may be absorbed into the body through the skin.

Component	Amount
Buffer A, pH 4.5 (Component A)	50 mL
Buffer B, pH 5.5 (Component B)	50 mL
Buffer C, pH 6.5 (Component C)	50 mL
Buffer D, pH 7.5 (Component D)	50 mL
Valinomycin (MW = 1111.33) (Component E)	5 mg
Nigericin, free acid (MW = 724.97) (Component F)	5 mg
DMSO (Component G)	1.3 mL

For research use only. Not for use in diagnostic procedures.

1. Dissolve Nigericin in 345 μ L anhydrous DMSO to make a 20 mM solution.
2. Dissolve Valinomycin in 225 μ L anhydrous DMSO to make a 20 mM solution.
3. Combine 100 μ L of Nigericin and 100 μ L of Valinomycin solutions to make a 1000 \times stock solution (10 mM each). Aliquot and store frozen for up to 1 year.
4. Dilute 10 μ L of this solution into 10 mL of the desired Intracellular pH Calibration Buffer to make a cell loading solution.
5. Perform desired cellular experiment with pH-sensitive pHrodo™ dyes or conjugates.
6. Wash cells 2 \times with Live Cell Imaging Solution (LCIS) (Cat. no. A14291DJ).
7. Replace LCIS with the cell loading solution, and incubate at 37°C for at least 5 minutes.
8. Analyze the cells using the appropriate Ex/Em maxima.
9. Repeat steps 7 and 8 with the three remaining Intracellular pH Calibration Buffers, if desired.

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