molecular probes®
by life technologies™

Rhod-3 Calcium Imaging Kit

Catalog no. R10145

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Rhod-3, AM (Component A); MW ~1600	1 vial	• 2°C–8°C • Desiccate • Protect from light	When stored as directed this kit is stable for at least 1 year
Dimethylsulfoxide (DMSO, Component B)	200 µL	• ≤25°C • Desiccate	
PowerLoad™ concentrate, 100X (Component C)	1 mL	• ≤–20°C	
Probenecid, water soluble (Component D)	2 × 77 mg	• ≤25°C • Desiccate	
Number of assays: Sufficient material is supplied for 10 assays based on the protocol below.			
Approximate fluorescence excitation/emission maxima: 550/580 in nm			
K_d for Ca^{2+}: 570 nM			

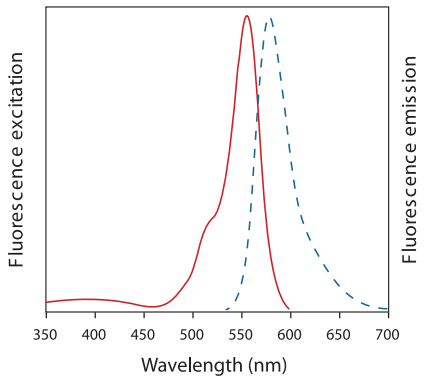
Introduction

Calcium indicators are important tools in signal transduction and cell-based pharmacological screening. As the intracellular and extracellular calcium gradient is vast (10–50 nM, and 1–3 mM, respectively, depending on the cell type), small changes in intracellular calcium [Ca^{2+}] can result in large cellular modifications.[1] In cells and tissues with blue or green autofluorescence[2], long-wavelength (*i.e.*, red-shifted) calcium indicators provide a means to bypass overlapping fluorescence and allow simultaneous monitoring of calcium activity. The red-shifted calcium dyes are also suitable for calcium imaging experiments multiplexed with green fluorescent protein (GFP) or other green fluorescent dyes.[3]

Rhod-3 AM, an improved red-shifted calcium dye, displays a more uniform cytosolic distribution and improved signal compared to existing red calcium dyes such as Rhod-2. The cationic nature of highly charged acetoxymethyl (AM) ester forms of calcium indicators results in potential-driven localized subcellular accumulation. However, imaging studies with the new Rhod-3 AM show minimal subcellular localization.

The cell permeant, nonfluorescent Rhod-3 AM is passively loaded into the cells in the presence of PowerLoad™ concentrate and probenecid, where the intracellular esterases cleave the dye to the cell impermeant, active form which fluoresces upon Ca^{2+} binding (Figure 1). PowerLoad™ concentrate, an optimized formulation of nonionic, Pluronic® surfactant polyols, aids the solubilization of Rhod-3 AM dye in physiological media.

Figure 1. Fluorescence excitation and emission spectra of Ca^{2+}-saturated Rhod-3 in pH 7.2 buffer.



PowerLoad™ concentrate is effective in combination with water soluble probenecid to aid AM ester dye-loading and retention in cells that actively extrude the de-acetylated dye form through anion pumps. Together, these reagents allow for maximal loading of dye with minimal effort in imaging applications yielding significantly larger fluorescent signal windows upon calcium binding when compared to existing red-shifted calcium dyes, as well as reduced subcellular compartmentalization.

Properties

Rhod-3 exhibits a large increase (>2.5 fold) in fluorescence upon binding Ca^{2+} and very low fluorescence at rest (without Ca^{2+} binding). To assess loading, use a maximal agonist concentration to reveal presence of the dye.

Rhod-3 has a dissociation constant (Kd) of 570 nM for Ca^{2+} determined at 22°C in 30 mM MOPS, pH 7.2 with 100 mM KCl. Kd values depend on pH, temperature, ionic strength and other factors, and are usually significantly higher in cellular environments.

Before you begin

Materials required but not provided

- Cell line and culture media of choice
- Physiological salt solution for loading and imaging such as phosphate buffered saline (PBS), HBSS (Hank's balanced salt solution), Ringers, or Krebs are acceptable depending on the cell type

Preparing Rhod-3 AM

Reconstitute the contents of Rhod-3 AM (Component A) vial in 100 µL of DMSO (Component B) to yield a stock solution of 10 mM Rhod-3 AM.
Store the 10 mM Rhod-3 AM stock solution desiccated at –20ºC, **protected from light in single use aliquots**. When stored as directed, this stock solution is stable for up to 3 months.

Preparing Probenecid

Dissolve the contents of one vial of Probenecid (Component D) in 1 mL of buffer, such as HBSS to prepare 250 mM Probenecid stock solution. Use the solution the same day or store at ≤–20°C for up to 6 months.

Buffers

For the cell loading protocol with Rhod-3 AM, you need to prepare three buffers as follows.

- **Loading buffer:** Prepare this buffer **fresh** as described in the protocol below and is composed of physiological buffer of choice containing 2.5 mM Probenecid (Component D), 1X PowerLoad™ concentrate (Component C), and 10 µM Rhod-3 AM.
- **Incubation buffer:** Used to incubate cells to allow complete cleavage of the AM esters and is composed of physiological buffer of choice containing 2.5 mM Probenecid (Component D). You need 2 mL of buffer for each loading.
- **Wash buffer:** Used to wash cells before and after AM ester loading and is the physiological buffer of choice.

Experimental protocol

The following protocol is designed for loading cells with 10 µM Rhod-3 AM in a total volume of 2 mL loading buffer and is optimized for loading adherent cells but is also suitable for loading suspension cells. Loading of CHO-M1, HeLa, and HEK-293 cells has been successfully demonstrated using this protocol.

For efficient loading of cells with Rhod-3 AM, we recommend including probenecid and PowerLoad™ concentrate in the loading buffer.

Loading protocol

1.1 To a 10–15 mL tube, add the following reagents in the order listed below to prepare **fresh** loading buffer:

100X PowerLoad™ concentrate (Component C)	20 µL
10 mM Rhod-3 AM	2 µL
Vortex to mix	
Physiological buffer of choice	to 2 mL
250 mM Probenecid (Component D)	20 µL
Invert the tube to mix.	

Note: If precipitation of Rhod-3 AM is observed, filter the loading buffer through a 0.2 µm filter prior to loading cells.

1.2 Remove media from adherent cells and wash cells twice in physiological buffer of choice.

Note: This loading protocol is also applicable for cells prepared in suspension. However, pellet cells by centrifugation when changing solutions to prevent loss of cells.

1.3 Add 2 mL loading buffer (from step 1.1) to cells and incubate cells in the **dark** at room temperature for 30–60 minutes.

1.4 Wash cells twice in physiological buffer of choice.

1.5 Add 2 mL incubation buffer (physiological buffer of choice containing 2.5 mM probenecid) and incubate cells at room temperature in the **dark** for 30–60 minutes.

1.6 Wash cells once in physiological buffer of choice. Cells are now ready for live-cell imaging.

Imaging

Image cells using a fluorescence microscope with standard rhodamine/TRITC filters. Fluorescence excitation and emission wavelength maxima for Rhod-3 AM are shown in Figure 1 (page 2). The following filters are recommended for fluorescence microscopy:

- Omega™ XF108, XF32 (www.omegafilters.com)
- Chroma™ 41002 or 31002 (www.chroma.com)

References

1. Nat Rev Mol Cell Biol. 7, 517 (2003); **2.** Anal Biochem. 291, 175 (2001); **3.** Biochem J. 356, 345 (2001).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
R10145	Rhod-3 Calcium Imaging Kit	1 kit
Related Products		
P10020	PowerLoad™ Concentrate, 100X	5 mL
P36400	Probenecid, water soluble	10 × 77 mg

Purchaser notification

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