molecular probes®
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Fluo-4 Calcium Imaging Kit

Catalog no. F10489

Table 1 Contents and storage

Material	Amount	Storage	Stability
Fluo-4, AM, 1000X in DMSO (Component A)	50 µL	• 2–8°C • Dessicate • Protect from light • DO NOT FREEZE	When stored as directed the product is stable for at least 1 year.
PowerLoad™ Concentrate, 100X (Component B)	500 µL	• 2–8°C • DO NOT FREEZE	
Neuro Background Suppressor, 10X (Component C)	5 mL	• 2–8°C • Protect from light • DO NOT FREEZE	
Probenecid, water soluble (Component D)	77 mg	• ≤25°C • Desiccate	

Number of assays: Sufficient material is supplied for 25 assays based on the protocol below.
Approximate fluorescence excitation and emission maxima: 494/506 nm (see Figure 1, page 2).
K_d for Ca^{2+}: 335 nM

Introduction

Changes in calcium gradients have been demonstrated to be important for several biological processes. As the intracellular and extracellular calcium gradient is vast (10–50 nM and 1–3 mM, respectively), small changes in intracellular calcium [Ca^{2+}] can result in large cellular modifications.[1] Visible light-excitable calcium indicators have been established as important tools for signal transduction and cell-based pharmacological testing. These small chemical entities (e.g., Fura-2, Indo, Fluo-3, and Fluo-4) display high sensitivity and a large fluorescent increase upon calcium binding.

The Fluo-4 Calcium Imaging Kit has been formulated, optimized, and contains all the necessary components for the detection of calcium flux by imaging applications. In addition to Fluo-4, AM, the kit contains PowerLoad™ Concentrate, a 10X Neuro Background Suppressor solution, and Probenecid.

For easy cell loading, the Fluo-4 Calcium Kit contains PowerLoad™ Concentrate. Due to its unique nature, PowerLoad™ solution can be used in the presence of complete culture medium, thus reducing the negative effects of replacing medium or loading in serum-free medium.

For Research Use Only. Not for use in diagnostic procedures.

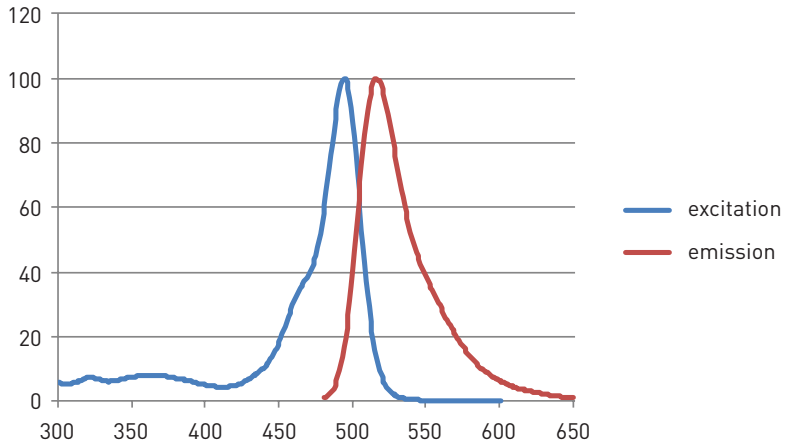
Baseline autofluorescence caused by components within the growth medium can be greatly reduced by the addition of the Neuro Background Suppressor solution. The Neuro Background Suppressor solution has been specifically formulated for use with neuronal cells and will not cause osmotic shock. Additionally, the Neuro Background Suppressor solution has been used successfully with many different cell types to efficiently suppress background fluorescence without sacrificing the specific cellular fluorescence generated in the assay.

A proprietary, water-soluble Probenecid (which is commonly used to inhibit cellular transport and thus reduce the baseline signal) is supplied with the Fluo-4 Calcium Imaging Kit.

Properties

- The emission from the calcium-bound Fluo-4 dye (excitation 494 nm/emission 506 nm) can be detected with standard FITC filters.
- Upon binding calcium the fluorescence intensity increases >100 fold.
- K_d for Ca^{2+} in buffer: ~335 nM

Figure 1 Fluorescence excitation and emission spectra of Ca^{2+}-saturated Fluo-4, AM in pH 7.2 buffer.



Before You Begin

Materials Required but Not Provided

- Cell line and culture medium of choice
- For optimal performance, we recommend Live Cell Imaging Solution (LCIS) (Cat. no. A14291DJ), a physiological buffered saline equivalent to Ringer's Solution.
- A sterile-filtered, 2 M Glucose Stock Solution will also be required for LCIS and many other solution formats to support cell health in longer term (hours) experiments, as well as studies with primary or differentiated neural cell types.
- Buffered and pH adjusted physiological saline solution for dye loading and imaging. Phosphate buffered saline (PBS), Hank's balanced salt solution (HBSS), Ringer's solution, or Krebs' solution are acceptable depending on cell type, and should be pH or osmotically adjusted for signaling or long term studies.

Prepare 20 mM Glucose + Live Cell Imaging Solution

Dilute 2 M Glucose Stock Solution 1:100 into LCIS for a final glucose concentration of 20 mM. Keep this solution clean and free of contaminants to prevent bacterial, fungal, or yeast growth once glucose has been added.

Prepare Probenecid

Dissolve the contents of the Probenecid (Component D) vial into 1 mL of LCIS to prepare the 100X Probenecid stock solution. Use the solution the same day or store at ≤–20°C for up to 6 months.

Experimental Protocols

The protocol below provides instructions for performing the calcium flux detection assay using adherent cells grown in a 35-mm dish with 2 mL of culture medium.

1.1 To a 15-mL tube, add the following reagents in the order listed below to prepare fresh Fluo-4, AM Loading Solution:

100X PowerLoad™ concentrate (Component B)	100 µL
Fluo-4, AM, 1000X (Component A)	10 µL

Vortex to mix.

Physiological buffer of choice or 20 mM Glucose Stock + LCIS	10 mL

Invert the tube to mix.

Optional: Add 100 µL of 100X Probenecid stock solution to prevent extrusion of cytosolic dye by anion pumps, which can decrease loading efficiency on some cell types.

1.2 Remove medium from adherent cells and wash cells once in physiological buffer of choice or LCIS.

1.3 Add 2 mL of Fluo-4, AM Loading Solution (from step 1.1) to cells, and incubate cells at 37°C for 15–30 minutes, followed by 15–30 minutes at room temperature.

Note: Cells may be loaded at room temperature for as long as 60 minutes.

1.4 Remove Fluo-4, AM Loading Solution, and wash cells once in physiological buffer of choice or LCIS.

1.5 Add 2 mL of physiological buffer of choice or 20 mM Glucose Stock in LCIS. Cells are now ready for live-cell imaging.

Optional: To suppress background fluorescence, add 1:10 diluted Neuro Backdrop Background Suppressor solution (Component C).

Imaging

Standard FITC settings may be used to visualize the cytosolic staining of the Fluo-4, AM dye. For a positive control, add Ionomycin (a calcium ionophore, Cat. no. I24222) to a final concentration of 10 µM for a large increase in cytosolic calcium concentration.

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
F10489	Fluo-4 Calcium Imaging Kit	1 kit
Related Products		
A14291DJ	Live Cell Imaging Solution	500 mL
I24222	Ionomycin, calcium salt	1 mg
14025-092	Hanks' Balanced Salt Solution (HBSS) (1X), liquid	500 mL
15630-106	HEPES Buffer Solution (1 M)	20 mL

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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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