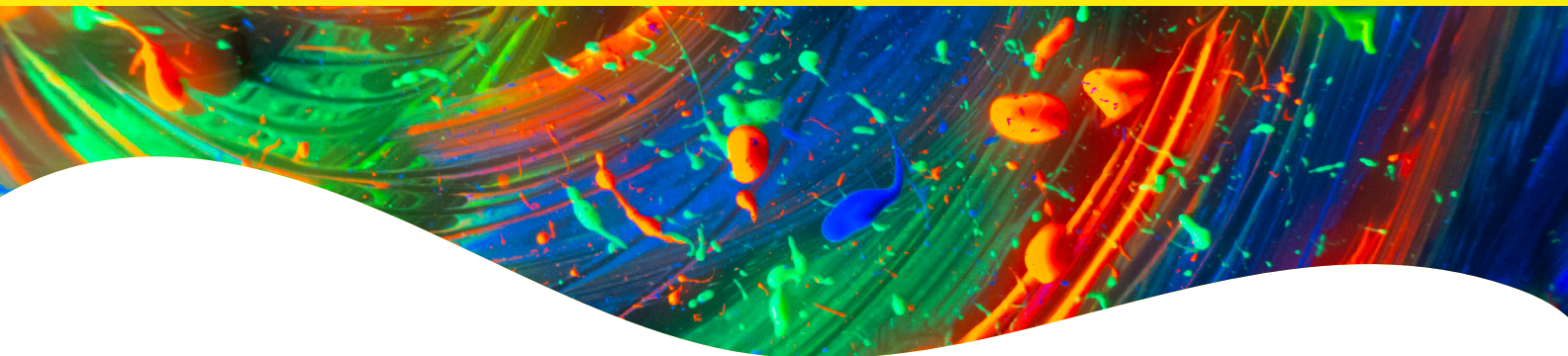


PhenoVue Fluo-4 AM, Calcium Indicator

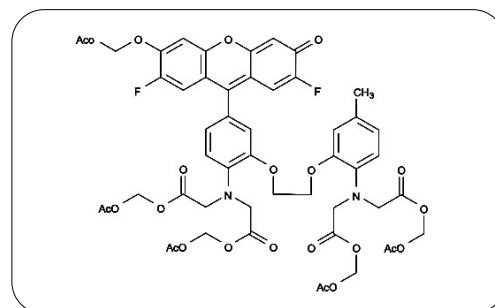


Overview

Calcium ions are important signaling molecules mediating a broad spectrum of intracellular, intercellular as well as extracellular functions. Ca^{2+} plays critical physiological functions such as muscle contraction, neuronal excitability, cell migration and cell growth. Intracellular Ca^{2+} is stored in the endoplasmic reticulum, sarcoplasmic reticulum, and the mitochondria. Upon cell surface receptors stimulation like GPCRs, calcium is released from these internal stores and its intracellular concentration increases from 100 nM to approximately 1 μM .

Intracellular Ca^{2+} responses have been widely investigated with fluorescent calcium probes whose fluorescent signal increases upon Ca^{2+} binding.

PhenoVue™ Fluo-4 AM, is a cell permeable Ca^{2+} indicator™ which is rapidly metabolized by intracellular esterase leading to a green, fluorescent signal.



Structure of PhenoVue Fluo-4 AM, calcium indicator. Source: PubChem CID 4060965

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Fluo-4 AM, calcium indicator	CP131	10	50 μg (45 nmol)	Solid	Dry ice

Storage and stability

- Store desiccated reagents at -16 °C or below, protected from light. Avoid repeated freeze / thaw cycles.
- The stability of these products is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.
- Allow the reagents to warm up to room temperature for 15 mins before opening the vials, and aliquot.
- Aliquoted reagents must be stored at -16 °C or below.

Equivalent number of microplates

Product name	When used at recommended concentration	96-well microplate (100 µL - 300 µL per well)	384-well microplate (25 µL - 90 µL per well)	1536-well microplate (4 µL - 12 µL per well)
PhenoVue Fluo-4 AM, calcium indicator	5 µM	Approx. 3 to 10	Approx. 3 to 10	Approx. 4 to 16

View our full range of high-quality imaging microplates at Revvity.com

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working solution*	Final concentration range
PhenoVue Fluo-4 AM, calcium Indicator	1096.95 g/mol	5 mM DMSO	20 µM	2-5 µM

* Dilutions can be done in PBS, HBSS, PhenoVue dye diluent A.

Notes:

- 0.04% PhenoVue pluronic F-127 nonionic detergent can be added to the working solution to improve Fluo-4 AM solubility.
- 4 mM PhenoVue probenecid solution can be added to the 20 µM working solution to inhibit organic anion-transporters and reduce leakage of de-esterified indicators, such as PhenoVue Fluo-4 AM.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Filter set	Epsilon (ϵ in $M^{-1}\cdot cm^{-1}$ at λ max)
PhenoVue Fluo-4 AM, calcium Indicator	495	528	FITC	82000

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue Fluo-4 AM, calcium Indicator	Yes	No	No

Protocols

Cell culture

Seed cells in imaging black wall, clear bottom microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO₂ until 50-70% confluency.

Staining

1. Add 2 to 5 µM Phenovue Fluo-4 AM final concentration to your cells. If needed, supplement your 20 µM working solution with 4mM Phenovue probenecid solution to prevent Phenovue Fluo-4 AM leakage.
2. Incubate at 37 °C, 5% CO₂ for 30 to 60 minutes. Depending on the cell type, incubation time can be extended to improve fluorescence signal intensity.
3. Replace the staining solution with fresh HBSS or any other appropriate medium, supplemented with Phenovue probenecid (1 mM final concentration) if needed.
4. Add your compound and simultaneously measure fluorescence using an imaging system such as the Opera Phenix® Plus high-content screening system.

Tips

- Phenovue pluronic F-127 20% solution is a non-ionic detergent that can be used to improve Phenovue Fluo-4 AM solubility.
- Phenovue probenecid solution is an inhibitor of organic anion-transporters that can be used to reduce leakage of de-esterified indicators, like Phenovue Fluo-4 AM.

Safety information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Applications

- High-content analysis/high-content screening
- Imaging microscopy

Validation data

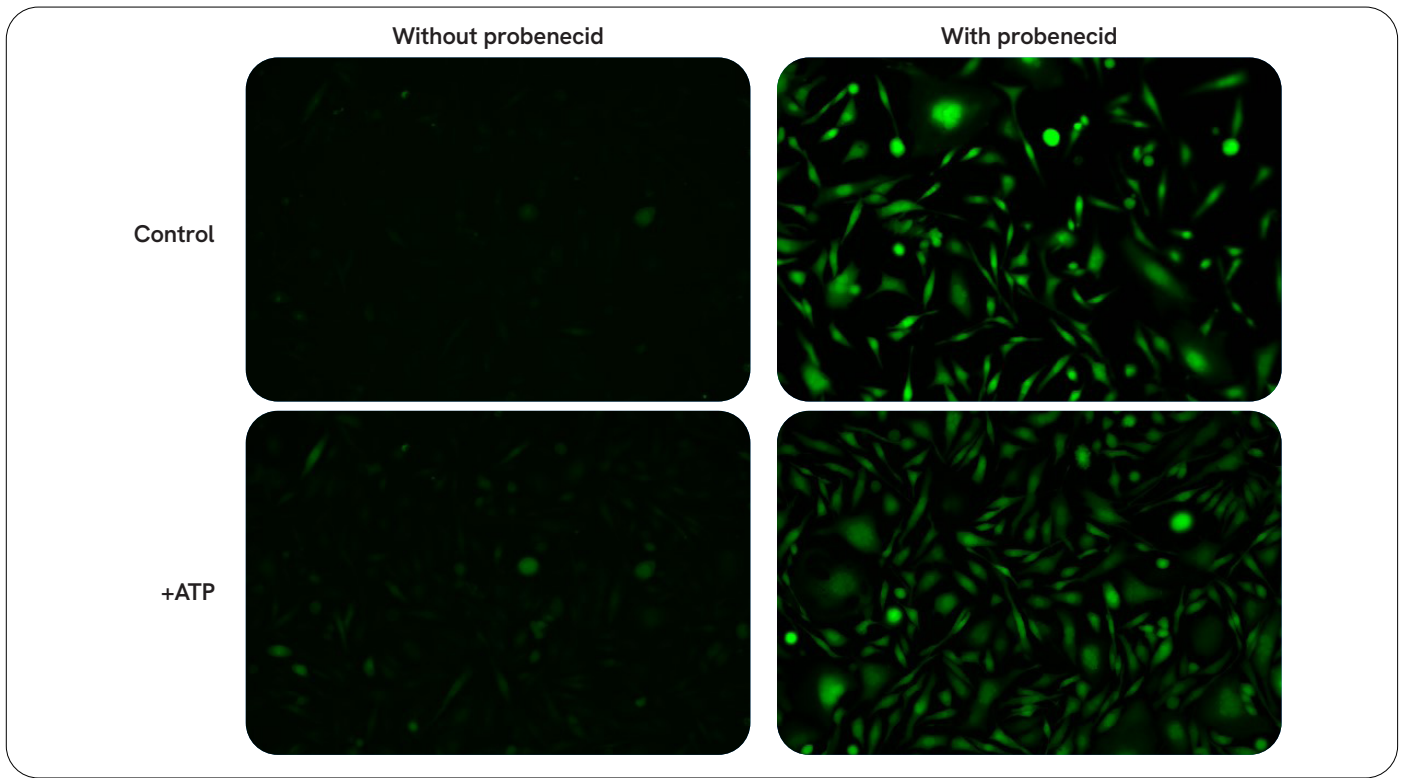


Figure 1: CHO-K1 cells were incubated at 37 °C for 45 min with **PhenoVue Fluo-4 AM** containing buffer in the presence or absence of 2.5 mM probenecid. ATP was added, and the images were acquired before (Control) and after (ATP) stimulation using FITC filter set.

