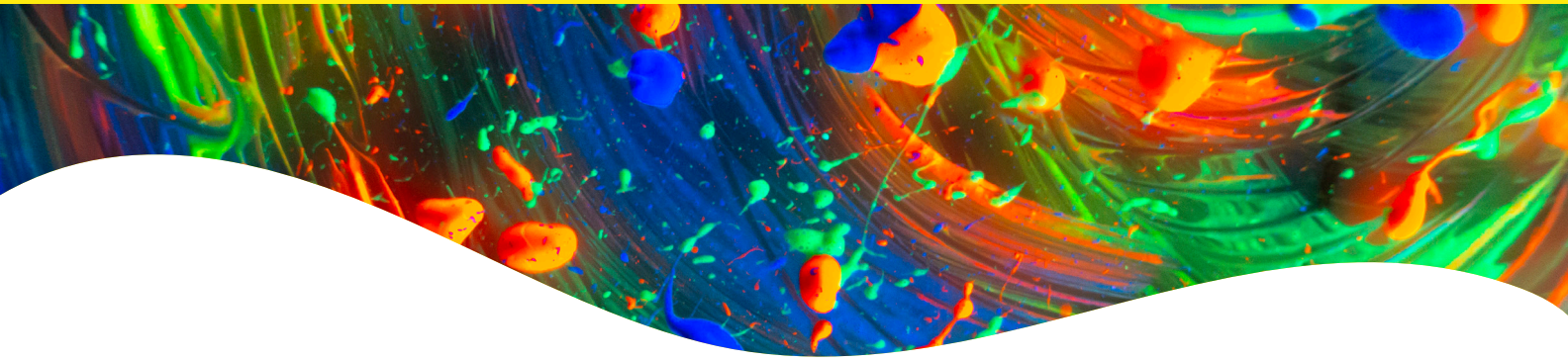
PhenoVue Cal AM Bright, Calcium Indicators (Cal-520 AM Bright and Cal-590 AM Bright)



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 which fluorescent signal increases upon Ca^{2+} binding.

PhenoVue™ Cal-520 AM Bright is a green, fluorescent calcium-sensitive dye which exhibits higher brightness, shorter incubation time, improved signal-to-noise ratio, reduced leakage and higher intracellular retention compared to other existing green calcium indicators, such as PhenoVue Fura-2, Fluo-4 AM and Cal-520 AM.

PhenoVue Cal-590 AM Bright is a red fluorescent calcium-sensitive dye which exhibits higher brightness, shorter incubation time, improved signal-to-noise ratio, reduced leakage, and higher intracellular retention, as well as homogeneous staining compared to other red calcium indicators such as Rhod-2 and PhenoVue Cal-590 AM. PhenoVue Cal-590 AM Bright is ideal for multiplexing experiments including FITC, PhenoVue Fluor 488 or GFP.

PhenoVue Cal-520 AM Bright and PhenoVue Cal-590 AM Bright are the most robust Ca^{2+} indicators.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Cal-520 AM Bright, calcium indicator	CP115201	10	50 µg (45 nmol)	Solid	Dry ice
PhenoVue Cal-590 AM Bright, calcium indicator	CP115901	10	50 µg (41 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 Cal-520 AM Bright, calcium indicator	5 µM	Approx. 3 to 10	Approx. 3 to 10	Approx. 4 to 16
PhenoVue Cal-590 AM Bright, 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 Cal-520 AM Bright, calcium indicator	1090.90 g/mol	5 mM DMSO	20 µM	2-5 µM
PhenoVue Cal-590 AM Bright, calcium indicator	1218.77 g/mol	5 mM DMSO	20 µM	2-5 µM

*Dilutions can be done in Hepes based buffer such as HBSS or PhenoVue dye diluent A.

Notes:

- 0.04% PhenoVue pluronic F-127 nonionic detergent can be added to the working solution to improve Cal-520 and Cal-590 solubility.
- 4 mM PhenoVue probenecid solution can be added to the 20 µM working solution to inhibit organic anion-transporters and further reduce leakage of de-esterified indicators, such as PhenoVue Cal-520 AM and PhenoVue Cal-590 AM, calcium indicators.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Filter set	Quantum yield
PhenoVue Cal-520 AM Bright, calcium indicator	493	515	FITC	75%
PhenoVue Cal-590 AM Bright, calcium indicator	581	593	Cy3/TRITC	nd*

*not determined

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue Cal-520 AM Bright, calcium indicator	Yes	No	No
PhenoVue Cal-590 AM Bright, 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% CO_2 until 50-70% confluency.

Staining

1. Add 2 to 5 µM PhenoVue Cal-520 AM Bright or Cal-590 AM Bright final concentration to your cells. If needed, supplement your 20 µM working solution with 4 mM PhenoVue probenecid solution to further improve calcium indicators intracellular retention.
2. Incubate at 37 °C, 5% CO_2 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 Calcium indicators solubility.
- PhenoVue probenecid solution is an inhibitor of organic anion-transporters that can be used to reduce leakage of de-esterified indicators.

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

PhenoVue Cal-520 AM Bright, calcium indicator

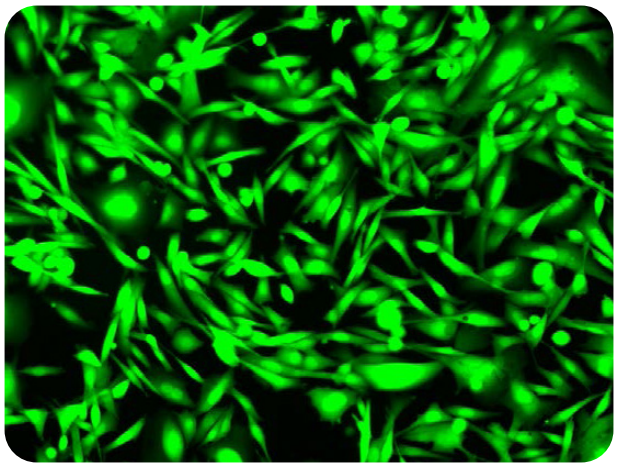


Figure 1: CHO-K1 cells were seeded in 96 well imaging microplates (50,000 cells/well). 9 µM **PhenoVue Cal-520 AM Bright** with 1 mM probenecid were added and the cells were incubated at 37 °C for 45 min. 100 µL fresh medium supplemented with 1 mM probenecid was added, then images were acquired using FITC filter set, after adding 10 µM ATP.

PhenoVue Cal-590 AM, calcium indicator

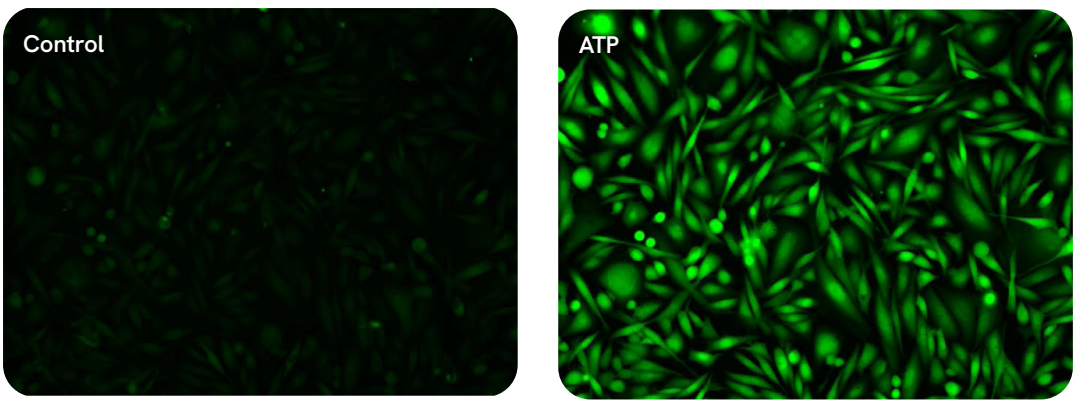


Figure 2: CHO-K1 cells were incubated at 37 °C for 45 min with **PhenoVue Cal-520 AM Bright** containing buffer in the absence of probenecid. ATP was added, and the images were acquired before (Control) and after (ATP) stimulation using FITC filter set.

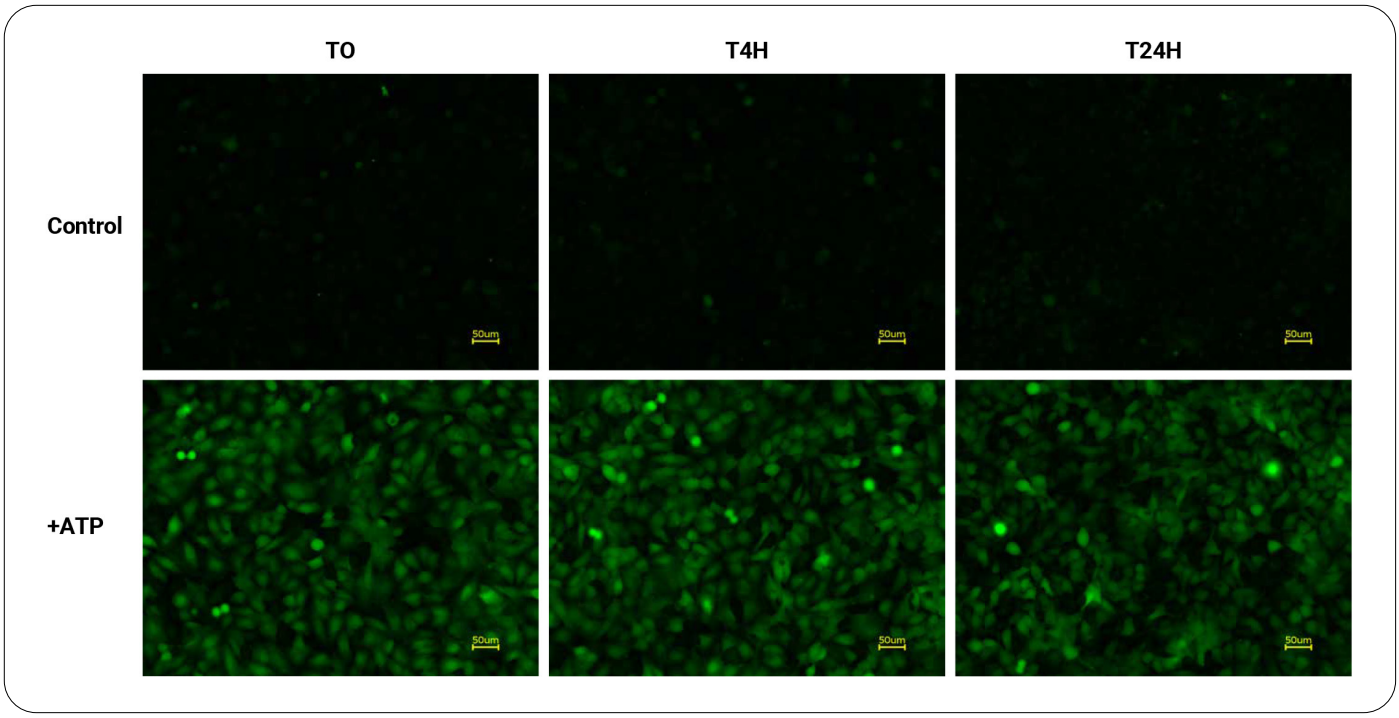


Figure 3: HeLa cells were stained with **PhenoVue Cal-520 AM Bright** in HHBS without probenecid, and the cells were incubated at 37 °C for 2 h. After staining, the cells were grown in the cell culture medium. At indicated time intervals, the cell culture medium was replaced with 200 µL HHBS, and 10 µM ATP were added before images acquisition using FITC filter set.

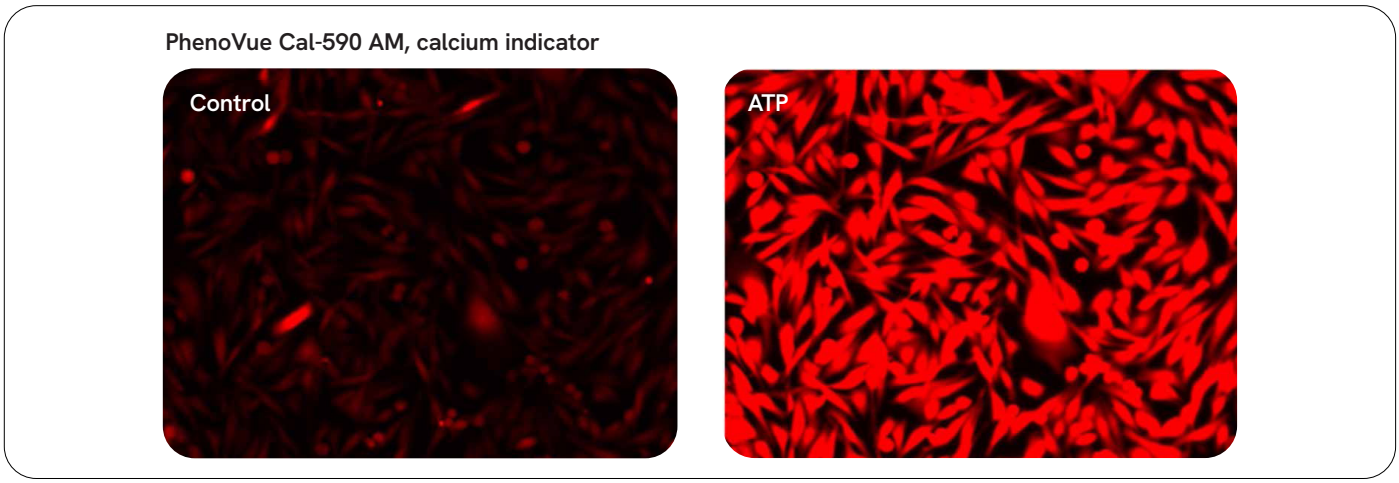


Figure 4: CHO-K1 cells were seeded in 96 well imaging microplates (40,000 cells/well). 100 µL of **PhenoVue Cal-590 AM Bright** with 1 mM probenecid were added and the cells were incubated at 37 °C for 60 min. 100 µL fresh medium was added, then images were acquired using Cy3/TRITC filter set, before and after. adding 50 µM ATP.

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