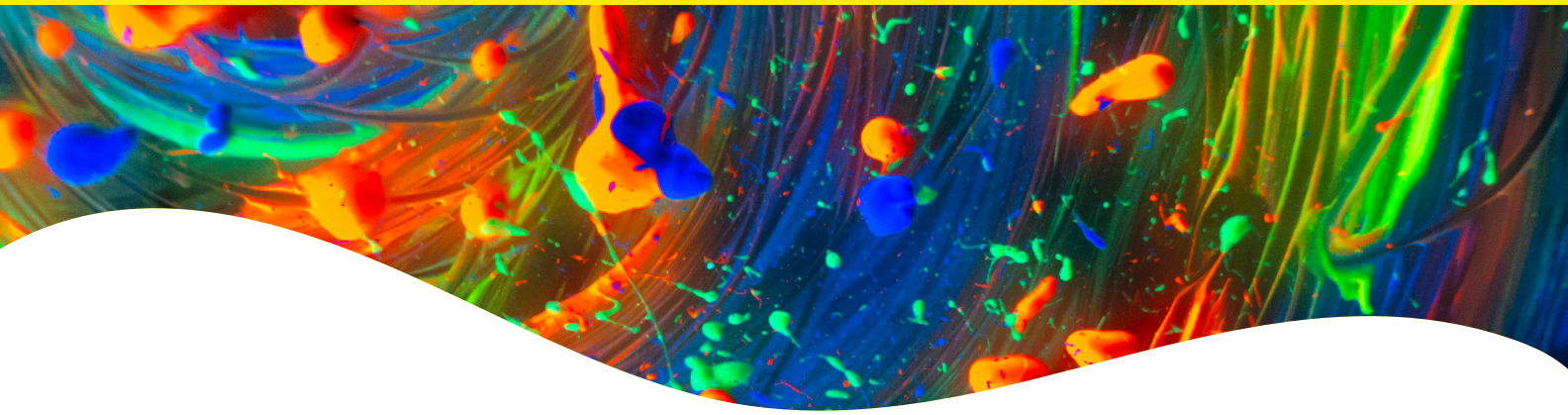
PhenoVue 492 live cell tracer stain



Overview

PhenoVue™ 492 Live cell tracer stain is a cell-permeable, non-fluorescent probe designed for long-term live-cell labeling. After passive entry into cells, the dye is activated by intracellular esterases, yielding a brightly fluorescent and membrane-impermeant form. Once converted, it reacts with intracellular thiol groups on proteins, generating stable covalent labeling that enables durable signal retention throughout extended live-cell experiments.

After a short loading period of 15–45 minutes, PhenoVue Fluor 492 enables reliable tracking of live cells for extended periods (up to 50–72 hours) without detectable cytotoxicity. The dye is highly photostable, is inherited by daughter cells during division, and provides bright green fluorescence. Its spectral properties make it fully compatible for multiplexing with blue, orange, and red fluorophores, offering flexibility for multicolor imaging applications.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue 492 Live cell tracer stain	CP23G1	1 vial	0.5 mg	Dried	Dry ice

Storage and stability

- Store stock solution at -16 °C or below, protected from light. Avoid repeated freeze/thaw cycles.
- The stability of this product is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.
- Allow the reagent to warm up to room temperature for 15 min before opening the vial and reconstitution.
- Aliquoted reagents must be stored at -16 °C or below and are stable for 4 months when using anhydrous DMSO.
- CAUTION: after reconstitution and freeze/thaw cycle, allow the reagent to warm up to room temperature for 30 min before taking an aliquot for your experiment. This will increase experiment-to-experiment reproducibility.

Reagent reconstitution and preparation of staining solutions

Reagent	1. Reconstitution	2. Preparation of staining solution (50µL/well) *
PhenoVue 492 Live cell tracer stain	Reconstitute with 54 µL anhydrous DMSO to give a 20 mM stock solution.	Dilute in cell culture medium to reach 0.5 to 15 µM

* Use supplemented cell culture medium compatible with your standard cell culture conditions.

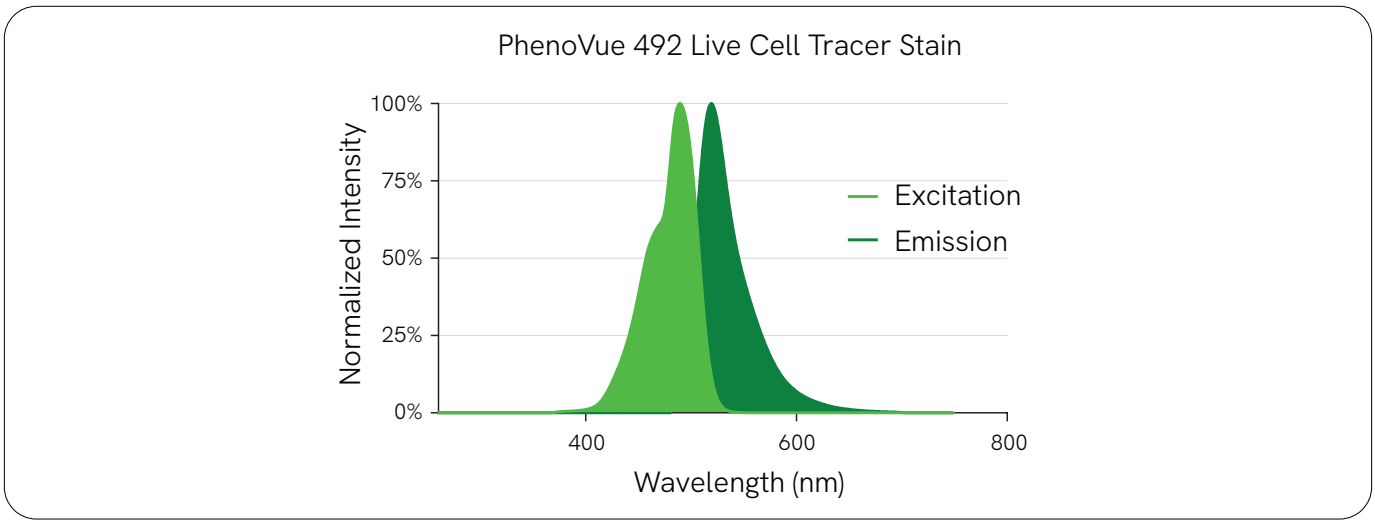
Recommended concentration

Product name	Stock concentration	Working concentration range*
PhenoVue 492 Live cell tracer stain	20 mM after reconstitution with 54 µL anhydrous DMSO.	Incubation < 24 h: 1 – 5 µM Incubation > 24 h: 5 – 15 µM Optimization range: 1 – 15 µM Start using 10 µM

Equivalent number of microplates

Product name	When used at concentration	96-well microplate (50 µL per well)	384-well microplate (25 µL per well)	1536-well microplate (12µL per well)
PhenoVue 492 Live cell tracer stain	10 µM	21	43	90

Spectral and photophysical properties



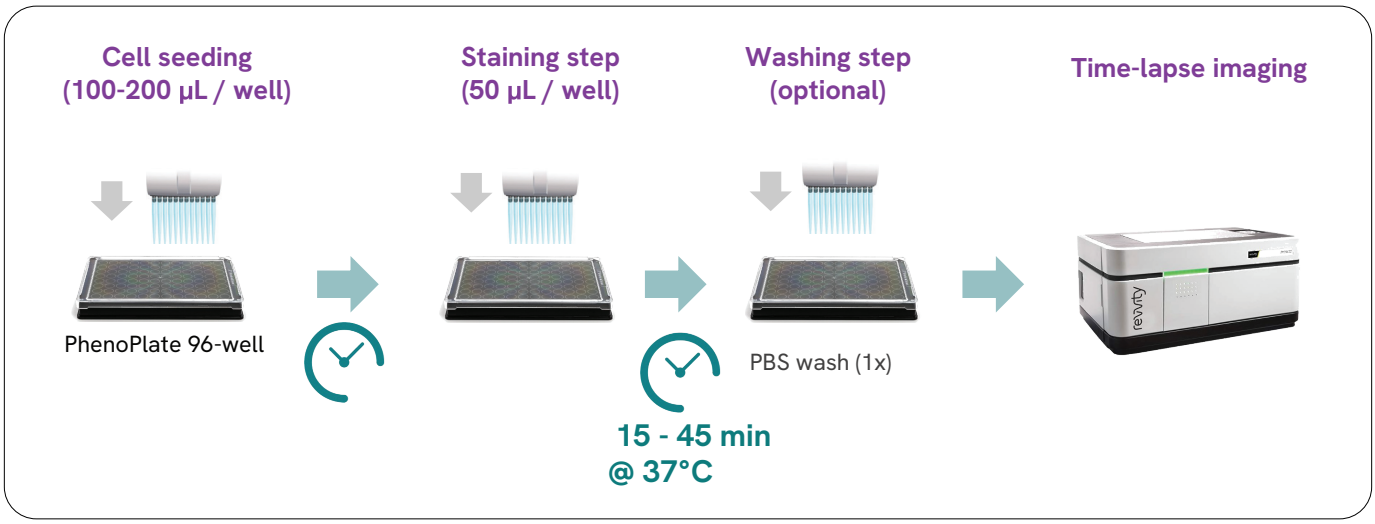
Product name	Maximum excitation/absorption wavelength (nm) *	Maximum emission wavelength (nm)*	Common filter set	Epsilon (ε in M-1.cm-1)**
PhenoVue 492 Live cell tracer stain	489/492	519	FITC	6000

* In aqueous buffer pH 10.6, simulating dye activation as the fluorogenic probe switches to its fluorescent state.
** in MeCN at 290nm

Live- and fixed-cell compatibility

Product name	Live cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue 492 Live cell tracer stain	Yes	No	No

Experimental workflow



Protocol for 96-well imaging plate

A 384-well microplate may also be used. In that case, adjust the cell seeding density appropriately and halve all reagent volumes.

Cell culture

Seed cells in PhenoPlate™ 96-well imaging microplates (or any other suitable cell culture vessel) and incubate under standard conditions (typically 37 °C, 5% CO_2). Optimize seeding density to ensure cells do not reach confluency at the final imaging time point.

Note: The PhenoVue 492 Live cell tracer stain is designed for use with live cells.

Live-cell imaging

1. **Remove** the culture medium.
2. **Incubate** cells with PhenoVue 492 Live cell tracer stain diluted in complete culture medium for **15–45 minutes** at 37 °C, 5% CO_2 (final volume 50–100 µL).

   For incubations **< 24 h:** use **2.5–10 µM**
   For incubations **> 24 h:** use **5–15 µM**
   Recommended optimization range: **2.5–15 µM**
3. **Remove** the dye solution.
4. **Optional rinse:** Wash once with 100 µL PBS to minimize background. Leaving residual dye can increase fluorescence background.
5. **Counterstaining (optional):** Add a nuclear marker such as PhenoVue Hoechst 33342 and/or a live-cell tracer for actin or tubulin (e.g. PhenoVue Fluor Actin or Tubulin Stains).
6. **Acquire images** using a live-cell imaging system. Time-lapse acquisition is recommended for tracking cell dynamics.

Tips

- A brief PBS rinse after dye loading is recommended to reduce background fluorescence, unless the dye solution is completely removed.
- Using **100 µL loading volume** can enhance fluorescence intensity at early time points.
- **Do not remove serum** during staining. Although serum-free conditions may enhance dye fluorescence, even short serum deprivation (≤ 45 min) can perturb cellular processes.
- Fluorescence gradually decreases over time but typically remains sufficient for cell **segmentation up to 50–72 hours**, depending on the cell type. The signal reduction is a combined result of **dye wash-out, and dilution during cell division.** To optimize signal retention, consider testing **PhenoVue probenecid** (part no. CP201) as an efflux pump inhibitor.
- Concentration, incubation time, and acquisition parameters should be **empirically optimized** for each model. Note that cytotoxic effects may appear with **excessive concentrations** or **high acquisition frequency/power.**
- To minimize cytotoxicity during long-term imaging, use the **lowest effective concentration**. In most cell types, **5–15 µM** provides strong signal and high-quality images without adverse effects.
- **PhenoVue Hoechst 33342** can also be cytotoxic at high doses. Lowering the concentration (e.g. ~100 ng/mL) usually provides adequate nuclear fluorescence with reduced toxicity.
- For multi-day live-cell experiments, refresh the culture medium regularly to maintain cell health.
- PhenoVue 492 Live cell tracer stain is photostable, enabling reliable **repeated acquisitions in time-lapse experiments.**

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
- Live-cell imaging

Recommendations for acquisition settings

For optimal fluorescent signal and image quality on a Revvity high-content screening system, we recommend the following acquisition settings, depending on the instrument used.

We recommend using confocal mode for image acquisition since it allows better image quality with higher signal over background.

Because cells are lived and dynamic, it is recommended to acquire 2 - 3 planes and apply the maximum projection for image analysis.

HCS instruments		PhenoVue 492 Live cell tracer stain
Opera Phenix™ Plus 5 lasers	Excitation laser (nm)	488
	Emission filters (nm)	500-550
Opera Phenix Plus 4 lasers	Excitation laser (nm)	488
	Emission filters (nm)	500-550
Operetta CLS 4 or 8 LED	Excitation LED (filters) (nm)	475 (460-490)
	Emission filters (nm)	500-550

Assay Validation

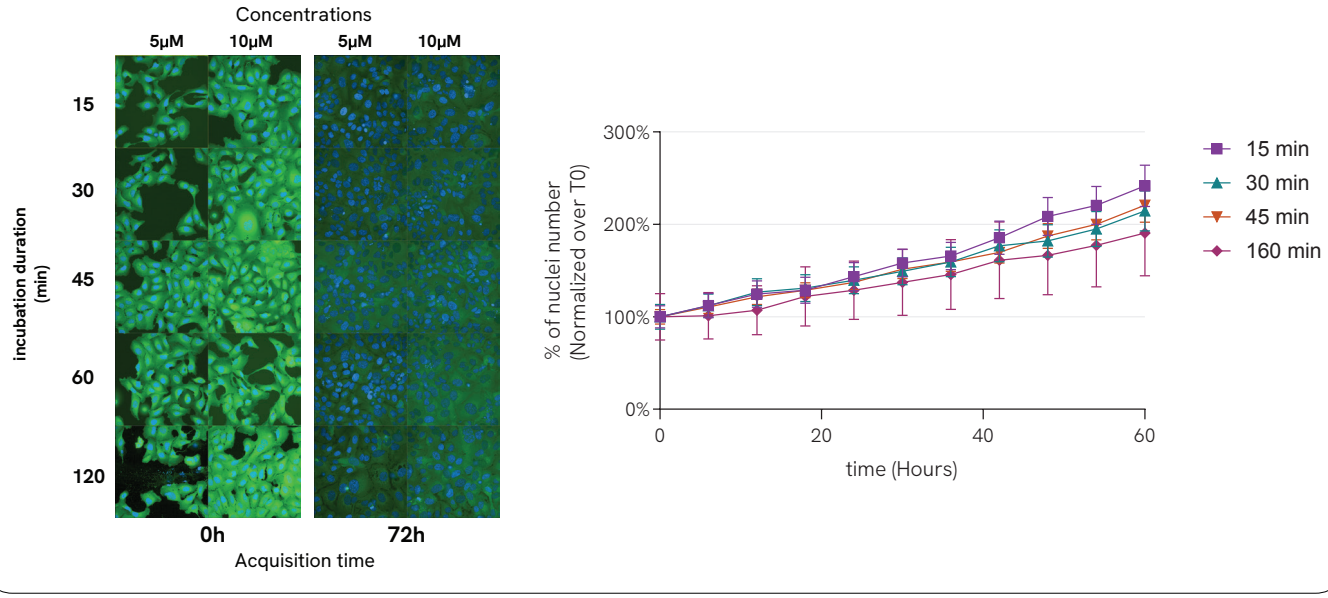


Figure 1: Effect of incubation duration on U2OS cell labeling and proliferation. Left: Representative fluorescence images at 0 h and 72 h. Right: Quantification of cell proliferation at 5 µM dye concentration over time, expressed as the percentage increase in nuclei number relative to T0.

Figure 1 shows U2OS cells (12,000 cells/well) seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (5 or 10 µM) for various incubation times (15 min, 30 min, 45 min, 1 h, 2 h), rinsed once with 100 µL PBS, and cultured in 200 µL of medium containing PhenoVue Hoechst 33342 (100 ng/mL). Images were acquired every 6 h on the Operetta CLS™ high-content analysis system using a water-immersion confocal objective (40x). Prolonged incubation (>45 min) increased dye uptake but did not further improve fluorescence intensity and slightly reduced cell proliferation, particularly after 2 h.

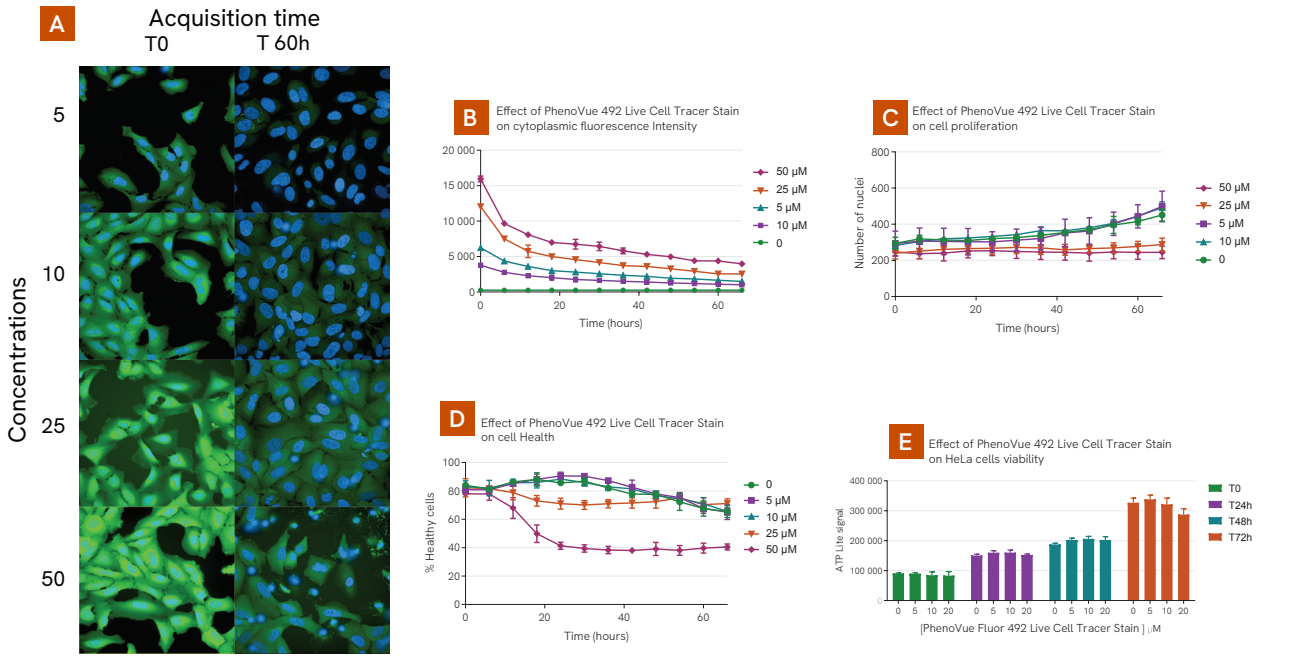


Figure 2: Dose-dependent effects of PhenoVue 492 Live cell tracer stain on U2OS and HeLa cells. Representative fluorescence images at T0 and T60 h for each concentration. Graphs represent quantitative analysis of U2OS cytoplasmic fluorescence intensity, proliferation, percentage of healthy cells over time, as well as cell viability in HeLa cells assessed by ATP-Lite assay.

Figure 2 shows U2OS cells (12,000 cells/well) seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (5, 10, 25, or 50 µM) for 45 minutes at 37 °C, rinsed once with 100 µL PBS, and cultured in 200 µL of medium containing PhenoVue Hoechst 33342 (100 ng/mL). Images were acquired every 6 h over 60 h using the Operetta CLS high-content analysis system with a water-immersion confocal objective (40x).

PhenoVue 492 Live cell tracer stain provided stable cytoplasmic fluorescence intensity over time at low-to-moderate concentrations (5–10 µM), enabling long-term monitoring of live cells. At these concentrations, U2OS cells maintained normal proliferation rates and high percentages of healthy nuclei, and HeLa cells preserved ATP levels up to 72 h. In contrast, higher concentrations (25–50 µM) led to reduced fluorescence stability, impaired proliferation, nuclear condensation, and a marked decrease in viability.

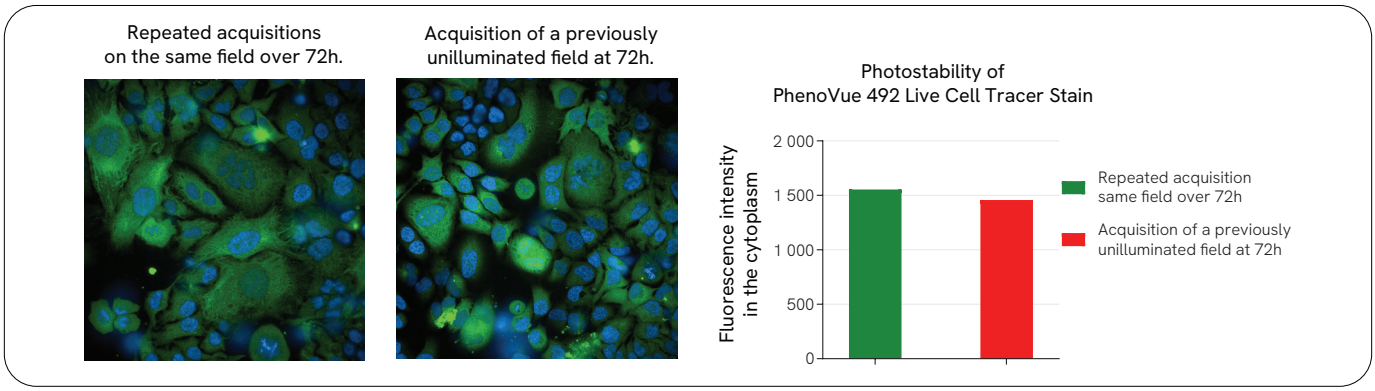


Figure 3: Photostability of PhenoVue 492 Live cell tracer stain. Left: images acquired after repeated or single field exposition after 72 h incubation. Right: Quantification of the fluorescence intensity in the cytoplasm after repeated or single field exposition after 72 h incubation.

Figure 3 shows A431 cells (15,000 cells/well) seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (20 µM, 45 min, 37 °C), rinsed once with 100 µL PBS, and cultured in 200 µL of medium containing PhenoVue Hoechst 33342 (100 ng/mL). Images were acquired on the Operetta CLS high-content analysis system every 6 h for up to 72 h (confocal, water-immersion objective, 40x, 140 ms exposure).

No significant loss of signal intensity was observed, indicating that PhenoVue Fluor 492 provides stable cytoplasmic fluorescence with minimal photobleaching under repeated long-term acquisition conditions.

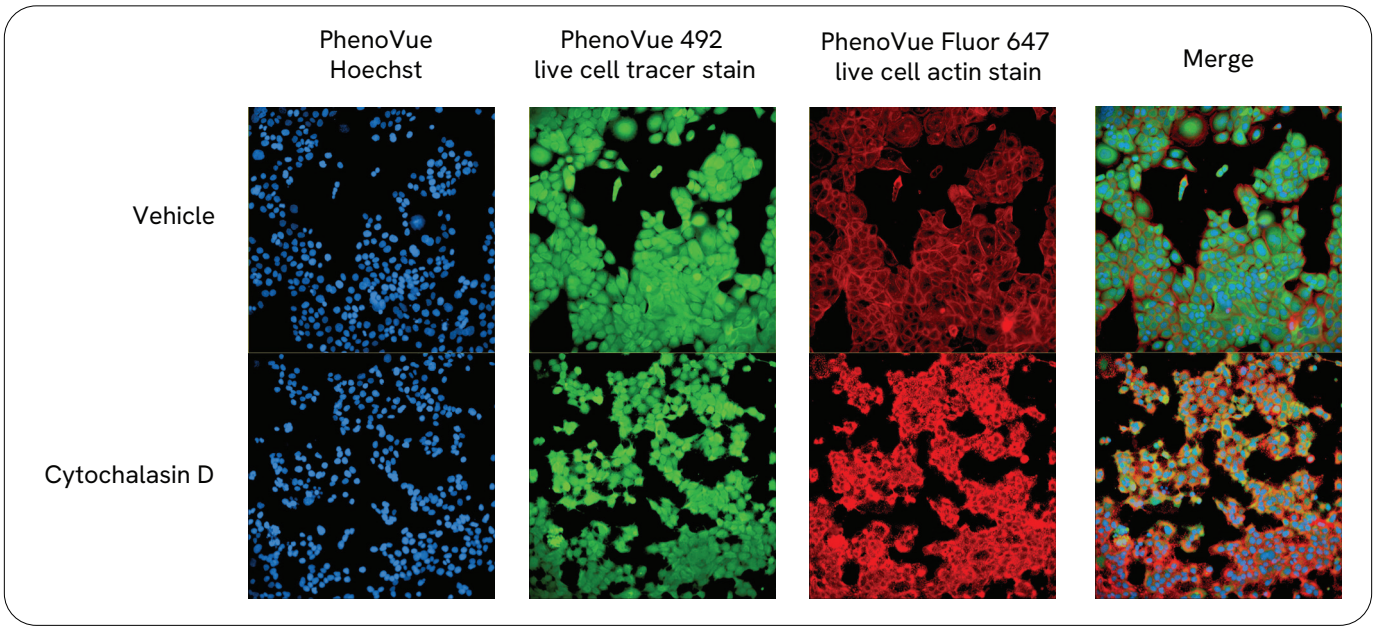


Figure 4: Compatibility of PhenoVue 492 Live cell tracer stain with PhenoVue Fluor 647 live-cell actin staining.

Figure 4 shows HaCaT cells (12,000 cells/well) seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (5 µM, 45 min, 37 °C), rinsed once with 100 µL PBS. After cell culture medium removal, wells were filled with 200 µL of medium containing PhenoVue Hoechst 33342 (100 ng/mL) and PhenoVue 647 Live cell actin stain (100 nM), with or without Cytochalasin D (2 µM). Images were acquired on the Operetta CLS high-content analysis system using a water-immersion confocal objective (20x).

PhenoVue Fluor 492 cytoplasmic staining (green) is compatible with Hoechst 33342 nuclear labeling (blue) and PhenoVue 647 live cell actin staiing (red), enabling multiplexed live-cell analysis of nuclear, cytoplasmic, and cytoskeletal dynamics.

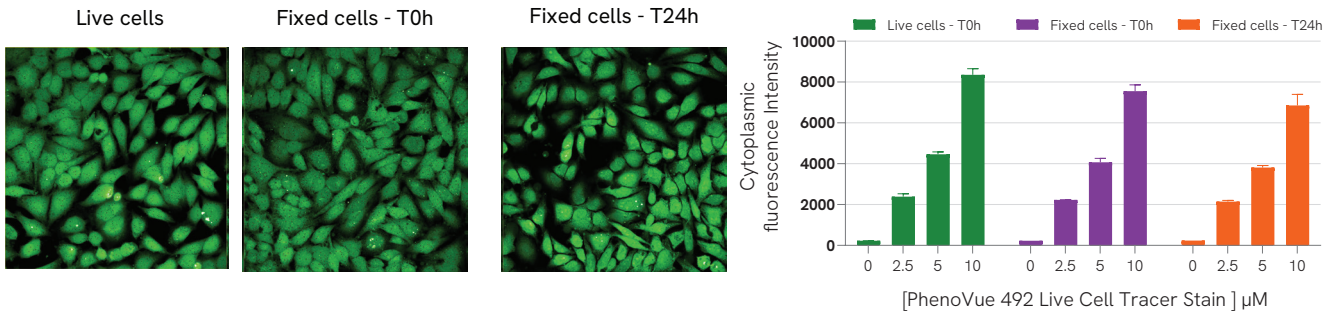


Figure 5: Fixability of PhenoVue 492 Live cell tracer stain across different concentrations. Left: Representative images of HeLa cells stained with 5 µM dye in live, T0-fixed, and T24 h-fixed conditions. Right: Quantification of cytoplasmic fluorescence intensity at different dye concentrations for live, T0-fixed, and T24 h-fixed samples.

Figure 5 shows HeLa cells (20,000 cells/well) seeded in PhenoPlate 96-well PDL-coated microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (2.5–10 µM, 45 min, 37 °C), rinsed once with PBS (100 µL), and either imaged live or fixed with PhenoVue PFA 4% for 10 min.

PFA fixation preserved PhenoVue 492 Live cell tracer stain-derived fluorescence but resulted in a progressive loss of signal intensity (10–30%) over 24 h, depending on dye concentration.

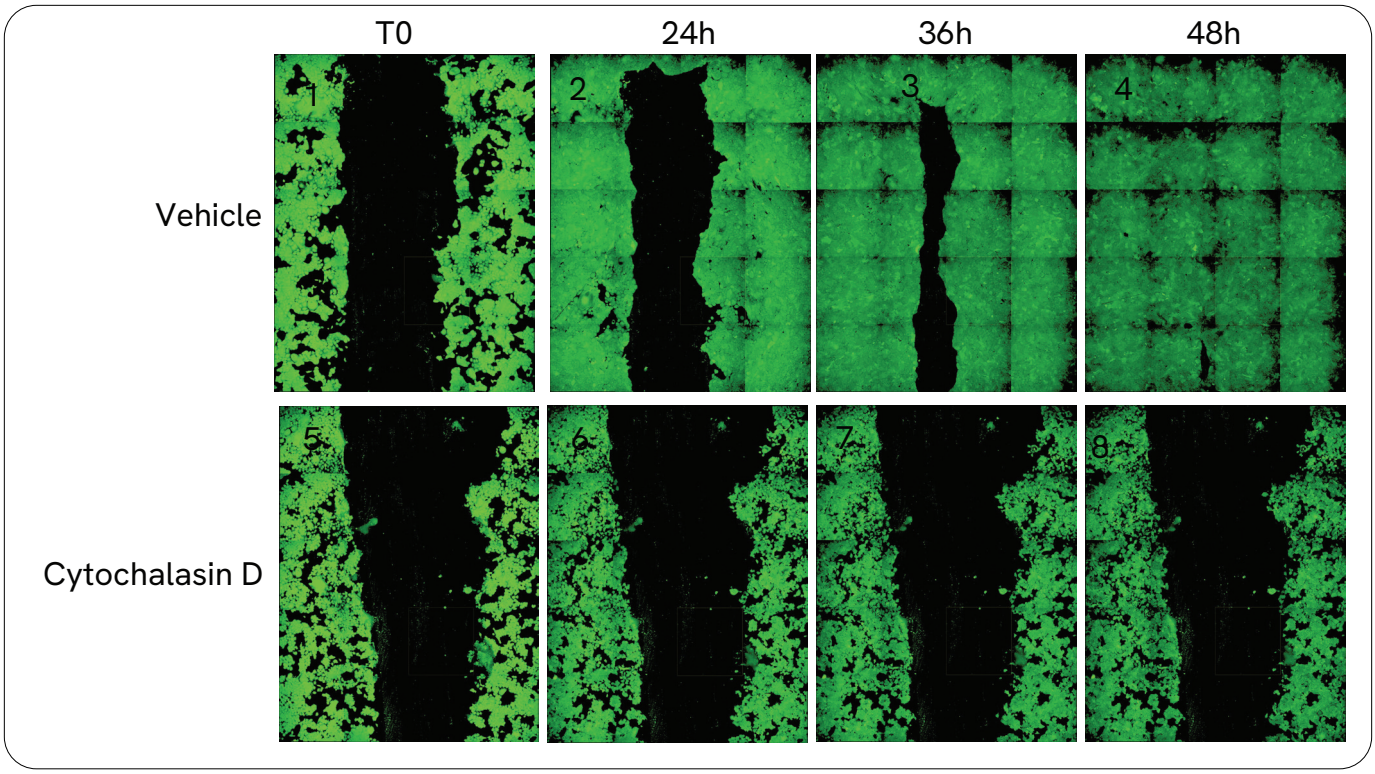


Figure 6: Time-lapse monitoring of HaCaT cell migration in a scratch assay using PhenoVue 492 Live cell tracer stain. Top row: Control condition, showing progressive closure of the scratch by migrating cells. Bottom row: Cytochalasin D (2 µM) treatment impaired cytoskeletal dynamics, resulting in reduced migration and delayed scratch closure.

Figure 6 shows HaCaT cells (12,000 cells/well) seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Cells were stained with PhenoVue 492 Live cell tracer stain (5 µM, 45 min, 37 °C), rinsed three times with 100 µL PBS, and a scratch was introduced using a multichannel pipette. After removal of floating cells, wells were refilled with 200 µL of medium containing PhenoVue Hoechst 33342 (100 ng/mL) with or without Cytochalasin D (2 µM). Images were acquired every 2 h for up to 48 h on the Operetta CLS Plus high-content screening system using a water-immersion confocal objective (20x).

PhenoVue 492 Live cell tracer stain enabled robust long-term visualization of HaCaT cell migration, clearly distinguishing normal wound closure from pharmacologically impaired motility.