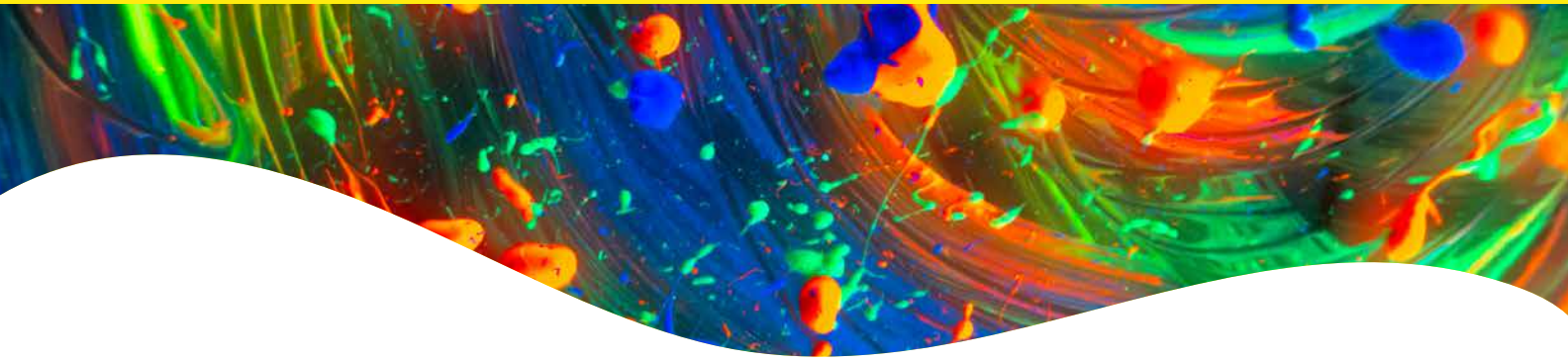
PhenoVue Fluor Live Cell Tubulin Stains



Overview

PhenoVue™ Fluor Live Cell Tubulin Stains are no wash, cell permeable fluorogenic dyes which specifically bind to polymerized tubulin and can be used to visualize microtubules in live cells. Sensitive, rapid and photostable, PhenoVue Fluor 647 Live Cell Tubulin Stain exhibits far-red emission and can be multiplexed with blue, green and orange colors, while PhenoVue Fluor 555 Live Cell Tubulin Stain exhibits orange emission and can be multiplexed with blue, green and far-red colors.

Like other tubulin stains derived from taxane, cytotoxicity can be observed with long exposure time (>24 h) which can be significantly limited by decreasing the concentration while maintaining high brightness and image quality (see recommended concentrations in the table below). Note that taxane derivatives such as Paclitaxel and Docetaxel which are commonly used chemotherapy inhibit microtubule depolymerization and cause cell cycle arrest. This phenomenon is also significantly limited while decreasing the concentration at concentration of PhenoVue Fluor Live Cell Tubulin Stain.

Depending on the cellular model, intracellular retention of PhenoVue Fluor Live Cell Tubulin Stain can be further improved in the presence of efflux pump inhibitor such as PhenoVue Probenecid, Ready to Use Solution or Verapamil.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Fluor 555 Live Cell Tubulin Stain	CP21O1	1	1x10 nmol	Desiccated	Dry ice
PhenoVue Fluor 647 Live Cell Tubulin Stain	CP21R1	2	2x50 nmol	Desiccated	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 6 months.
- **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.

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working concentration range*
PhenoVue Fluor 555 Live Cell Tubulin Stain	1300 g/mol	Reconstitute with 50 µL anhydrous DMSO to give a stock concentration of 0.2 mM	Incubation < 10h: 50 – 300 nM Incubation > 10h: 1 – 10 nM Optimization range: 1 – 300 nM
PhenoVue Fluor 647 Live Cell Tubulin Stain	1400 g/mol	Reconstitute with 50 µL anhydrous DMSO to give a stock concentration of 1 mM	Incubation < 10h: 300 – 1,000 nM Incubation > 10h: 50 – 300 nM Optimization range: 50 – 1 000 nM

* Dilutions can be done in cell culture medium.

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 Fluor 555 Live Cell Tubulin Stain	50 nM	6 to 20	6 to 20	10 to 32
PhenoVue Fluor 647 Live Cell Tubulin Stain	300 nM	5 to 17	5 to 17	9 to 27

See our range of high-quality imaging microplates here.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Common filter set	Epsilon* (ε in $M^{-1}.cm^{-1}$ at λ max)
PhenoVue Fluor 555 Live Cell Tubulin Stain	558	580	Cy3	75,000
PhenoVue Fluor 647 Live Cell Tubulin Stain	650	670	Cy5	140,000

* In HBSS + 0.2% SDS (Because the probe is fluorogenic, SDS is mandatory to mimic tubulin binding and switch on the dye absorbance and fluorescence).

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue Fluor 555 Live Cell Tubulin Stain	Yes	No	No
PhenoVue Fluor 647 Live Cell Tubulin Stain	Yes	No	No

Note: PhenoVue Fluor Live Cell Tubulin Stains are cell permeable. Therefore, these dyes are compatible with live cells but cannot be fixed after staining or used with fixed samples.

Protocols

Cell culture

Seed cells in imaging 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.

Note: PhenoVue Fluor Live Cell Tubulin Stains are compatible with live cells only. They cannot be fixed after staining or used with fixed samples.

Live-cell imaging

1. Remove cell culture medium.

2. Incubate with PhenoVue Fluor Live Cell Tubulin Stain in cell culture medium for 30 min at 37 °C, 5% CO_2.

   PhenoVue Fluor 647 Live Cell Tubulin Stain:
   < 10h incubation: 300 – 1000 nM
   >10h incubation: 50 – 300 nM
   Optimization range: 50 – 1000 nM

   PhenoVue Fluor 555 Live Cell Tubulin Stain:
   < 10h incubation: 50 – 300 nM
   >10h incubation: 1 – 10 nM
   Optimization range: 1 – 300 nM

   A nuclear staining dye, such as PhenoVue Hoechst 33342, can be mixed with PhenoVue Fluor Live Cell Tubulin Stains.

3. Acquire images on a live-cell imaging device.

Recommendations

- We recommend not to remove the cell culture medium containing the PhenoVue Fluor Live Cell Tubulin Stain prior to the time lapse acquisition (no wash experiment). As the dyes are fluorogenic, they become fluorescent only when bound to tubulin and display very low fluorescence background when free in medium or cells.
- We recommend no-wash experiments. PhenoVue Fluor Live Cell Tubulin Stain binds tubulin in a dynamic manner (rapid on/off rates). Thus, introducing washing steps can lead to fluorescent signal decrease until complete staining wash out.
- Concentration, incubation time, and acquisition settings of PhenoVue Fluor Live Cell Tubulin Stain should be optimized depending on the cellular model studied.
- Note that cytotoxicity of staining reagents, such as Tubulin probes, is usually observed in long term imaging and is especially accentuated with high acquisition frequency and power.
- To limit cytotoxic effects, we recommend using PhenoVue Fluor Live Cell Tubulin Stains at low concentration. Usually concentrations between 50 and 300 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain or between 1 and 10 nM of Phenovue Fluor 555 Live Cell Tubulin Stain result in high brightness and image quality. PhenoVue Hoechst 33342 can also lead to cytotoxicity. This can be reduced by using a lower concentration, such as 0.1 µg/mL which usually gives acceptable fluorescence.
- For live cell experiments over several days, it is recommended to replace cell culture medium supplemented with PhenoVue Fluor Live Cell Tubulin Stains every day.
- Depending on the cellular model, the addition of efflux pump inhibitor, such as probenecid, improves intracellular retention of PhenoVue Fluor Live Cell Tubulin Stains while enabling strong staining. Supplement cell culture medium, containing the dye, with PhenoVue Probenecid at 2.5 mM (0.77 mg/mL) or Verapamil at 10 µM.
- The PhenoVue Fluor Live Cell Tubulin Stain is based are taxol analog conjugates. Then compounds binding onto tubulin on taxol binding site will impair the fluorescent probe staining.

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

Validation data

PhenoVue Fluor 647 Live Cell Tubulin Stain

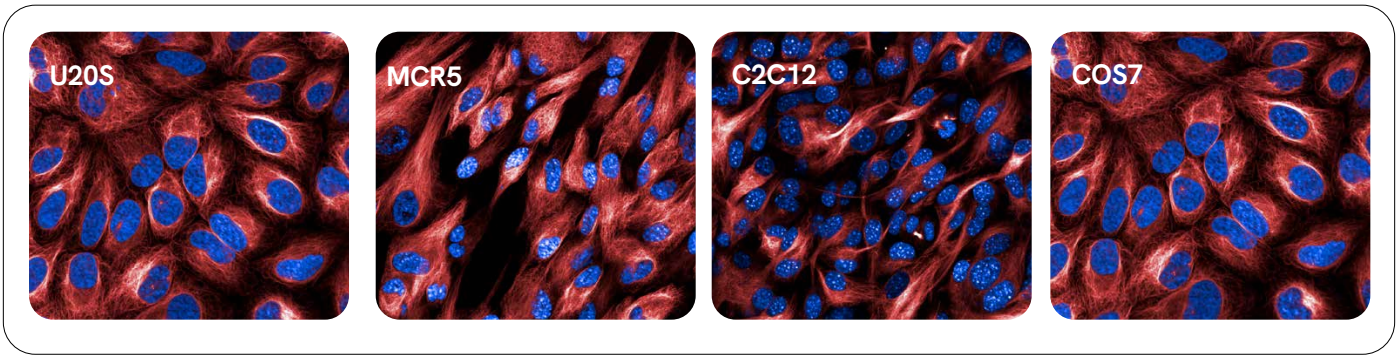


Figure 1: U2OS cells (15,000 cells/well); MRC5 cells (30,000 cells/well); C2C12 cells (40,000 cells/well) and COS7 (15,000 cells/well) were seeded in PhenoPlate™ 96-well microplates and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 300 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 for 4 h 30 min at 37 °C, 5% CO_2. For COS7 cell line only, the probes were co-incubated with 2.5 mM (0.77 mg/mL) PhenoVue Probenecid to inhibit efflux pumps and allow efficient staining. Other cell lines did not require efflux pump inhibitor. Images were acquired on the Opera Phenix® Plus high-content screening system. 63X water objective confocal.

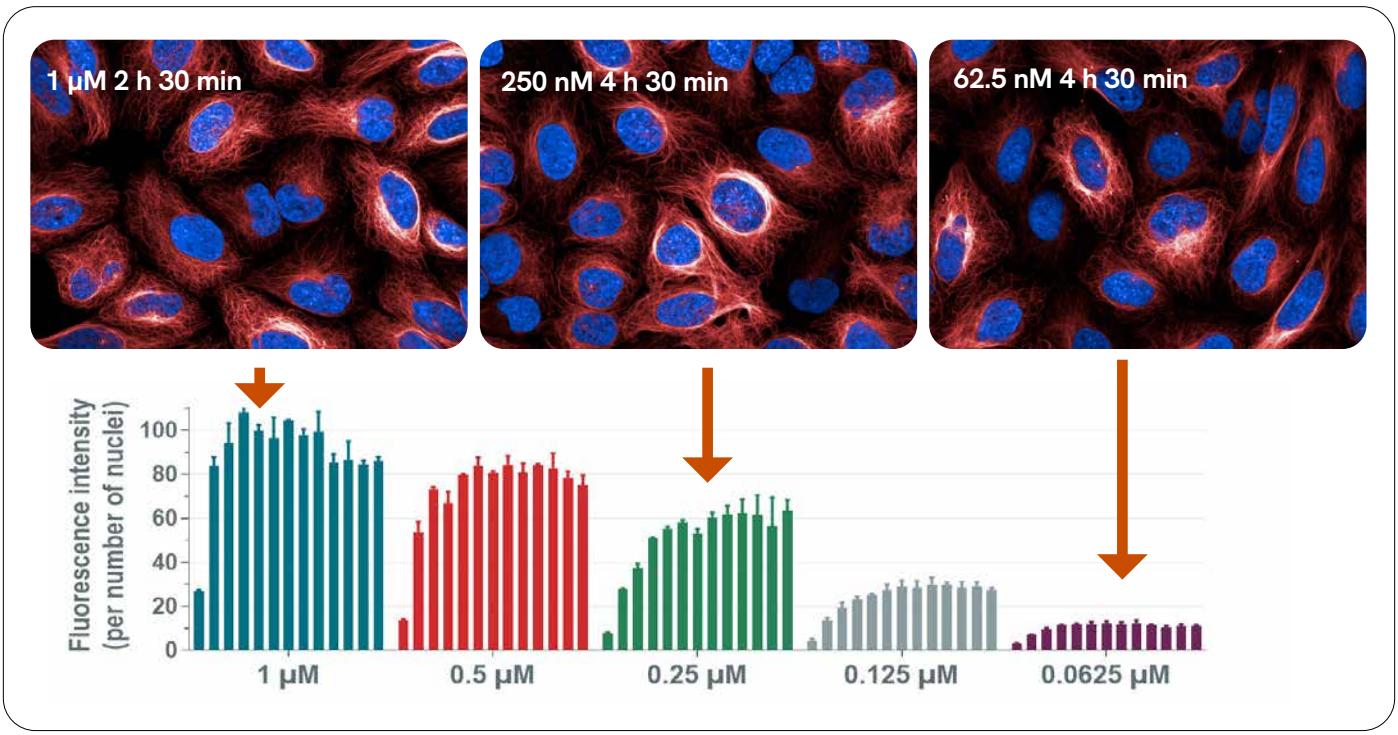
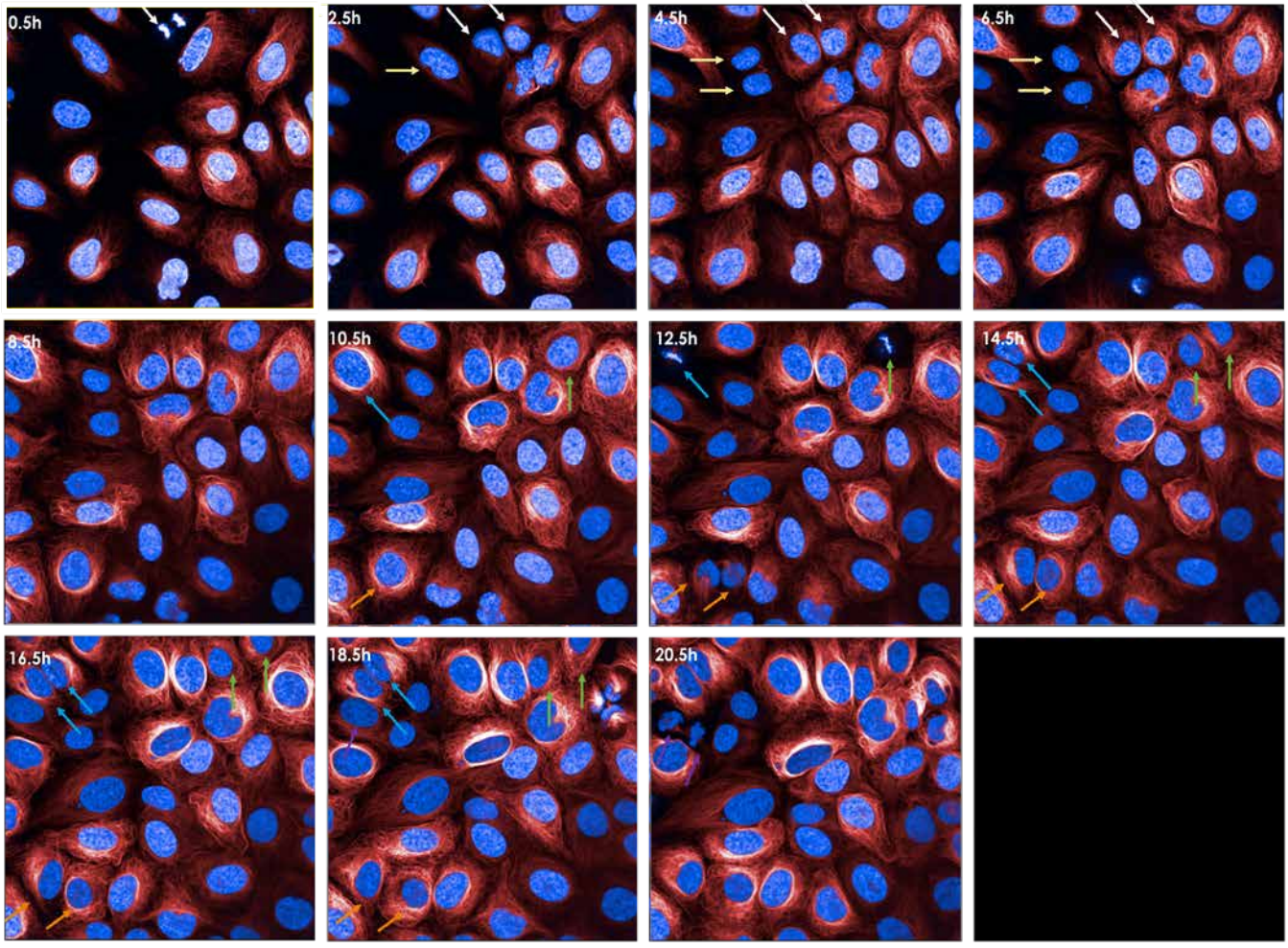


Figure 2: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 62.5-1,000 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 and incubated at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system every 2 h for 24 h. 63X water objective confocal. The histogram shows the fluorescence quantification for each concentration of PhenoVue Fluor 647 Live Cell Tubulin Stain over time.



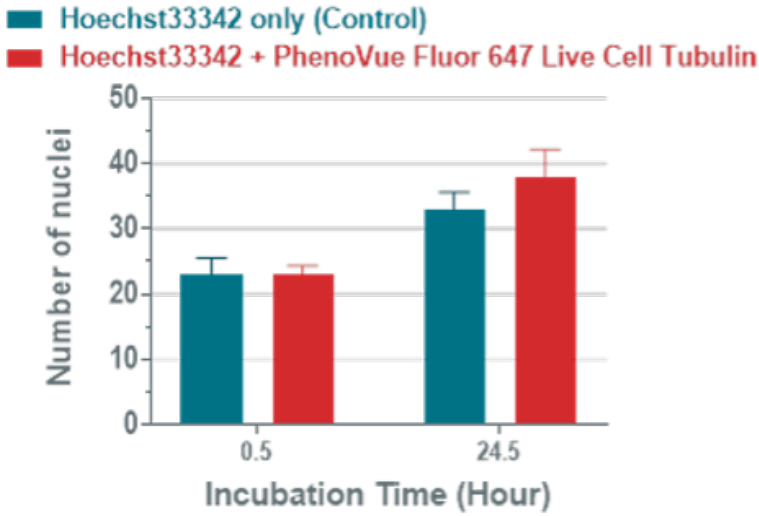


Figure 3: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 300 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 and incubated at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system every 2 h for 20 h. 63X water objective confocal. Cell division and proliferation is not impaired by PhenoVue Fluor 647 Live Cell Tubulin Stain, as determined by the quantification of nuclei displayed on the histogram.

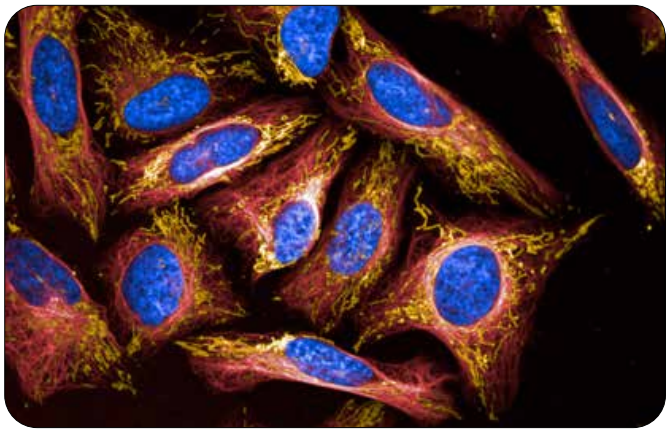


Figure 4: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 55 nM PhenoVue 561 Mitochondrial Stain (Orange), 300 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain (Red) and 0.125 µg/mL PhenoVue Hoechst 33342 (Blue) and incubated 1 h at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system. 63X water objective confocal. PhenoVue Fluor 647 Live Cell Tubulin Stain can be multiplexed with standard blue, green and orange channel dyes.

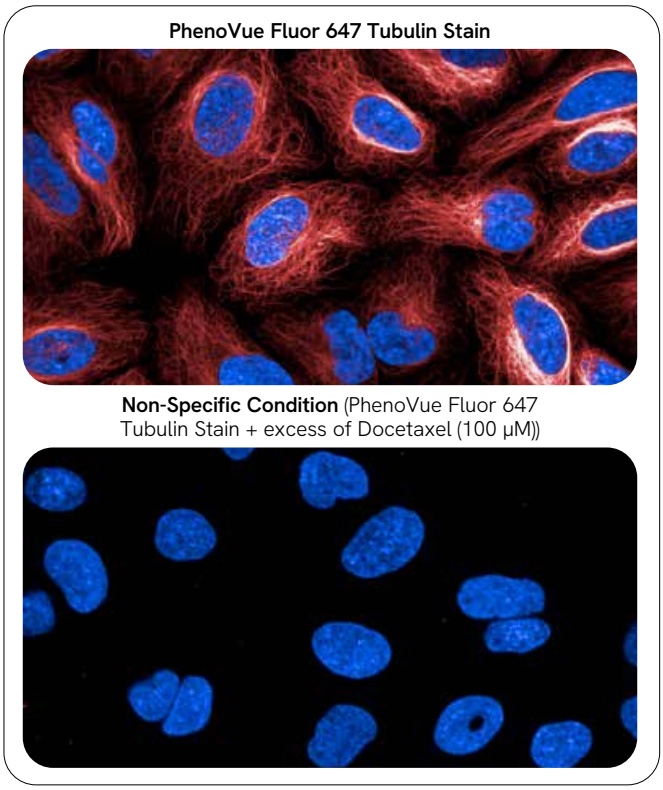


Figure 5: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then incubated with cell culture medium alone (top panel) or 100 µM docetaxel (tubulin specific taxane compound – bottom panel) for 30 min. Cells were then stained with 1,000 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain (Red) + 0.125 µg/mL PhenoVue Hoechst 33342 (Blue) and incubated 2 h 30 min at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system. 63X water objective confocal. PhenoVue Fluor 647 Live Cell Tubulin Stain, based on taxane derivative conjugated to a red dye, stain specifically polymerized tubulin as shown by docetaxel complete inhibition.

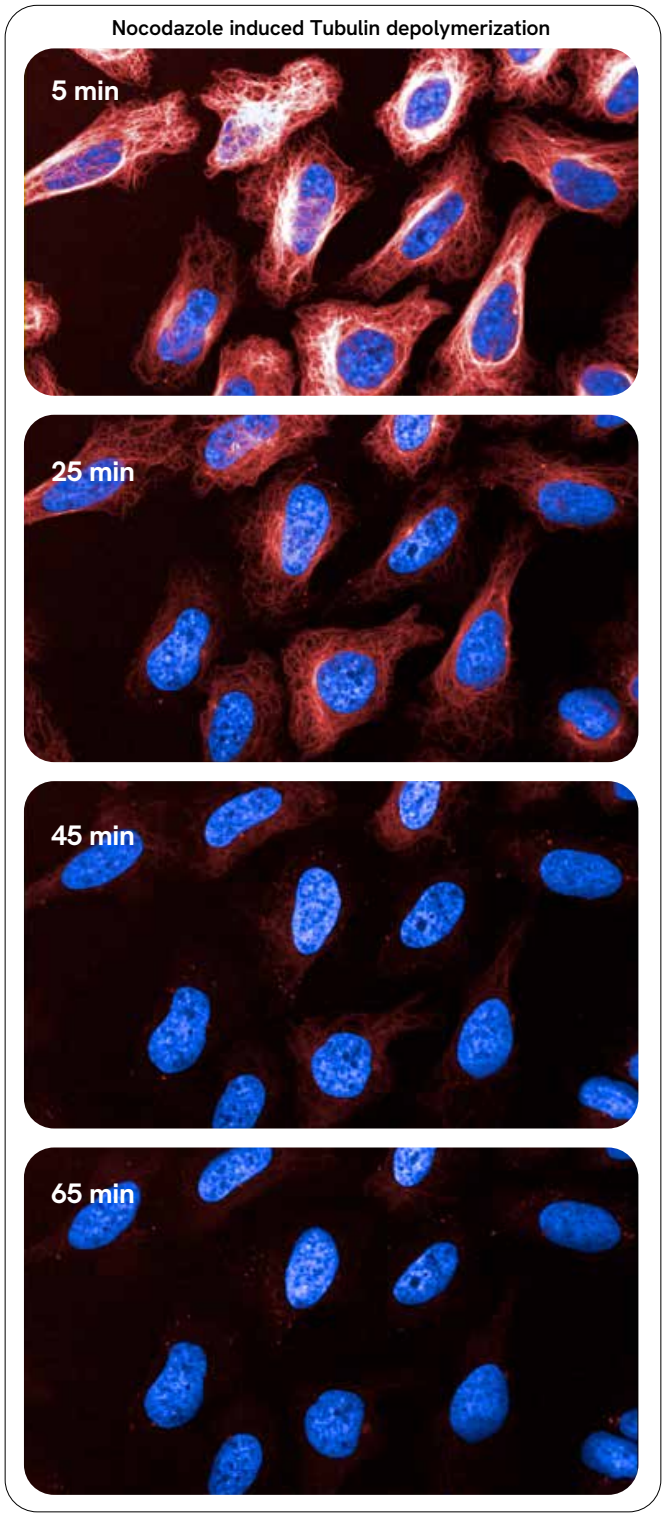


Figure 6: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 1,000 nM of PhenoVue Fluor 647 Live Cell Tubulin Stain (Red) + 0.125 µg/mL PhenoVue Hoechst 33342 (Blue) for 45 min at 37 °C, 5% CO_2. Nocodazole (10 µM), a tubulin depolymerization compound, was then added. Images were acquired on the Opera Phenix Plus high-content screening system in Time Lapse with the 63X water objective in confocal mode. PhenoVue Fluor 647 Live Cell Tubulin Stain allow to monitor nocodazole-induced tubulin depolymerization.

PhenoVue Fluor 555 Live Cell Tubulin Stain

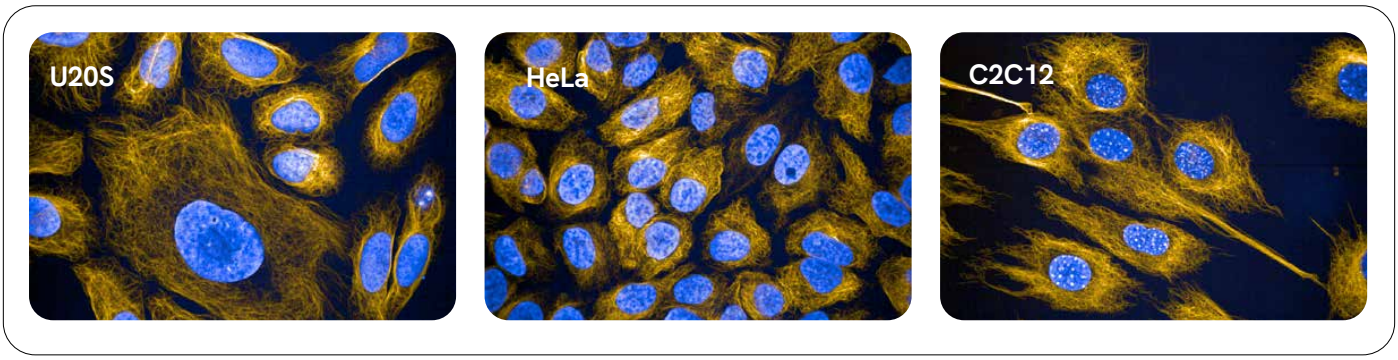


Figure 7: U2OS cells (15,000 cells/well); HeLa cells (40,000 cells/well) and C2C12 cells (20,000 cells/well) were seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 4 nM (U2OS) or 100nM (HeLa and C2C12) of PhenoVue Fluor 555 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 for 30 min at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system. 63X water objective confocal.

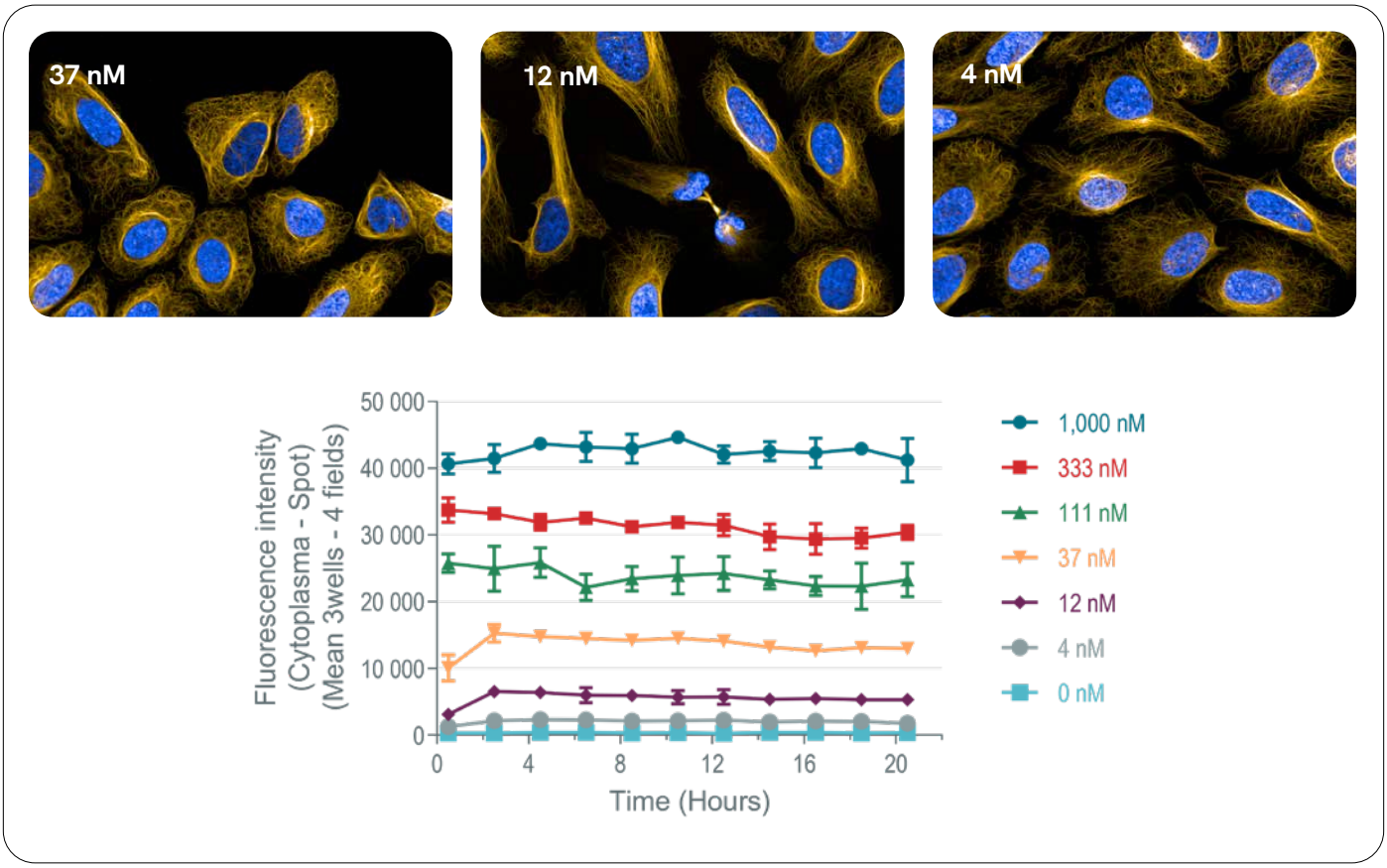
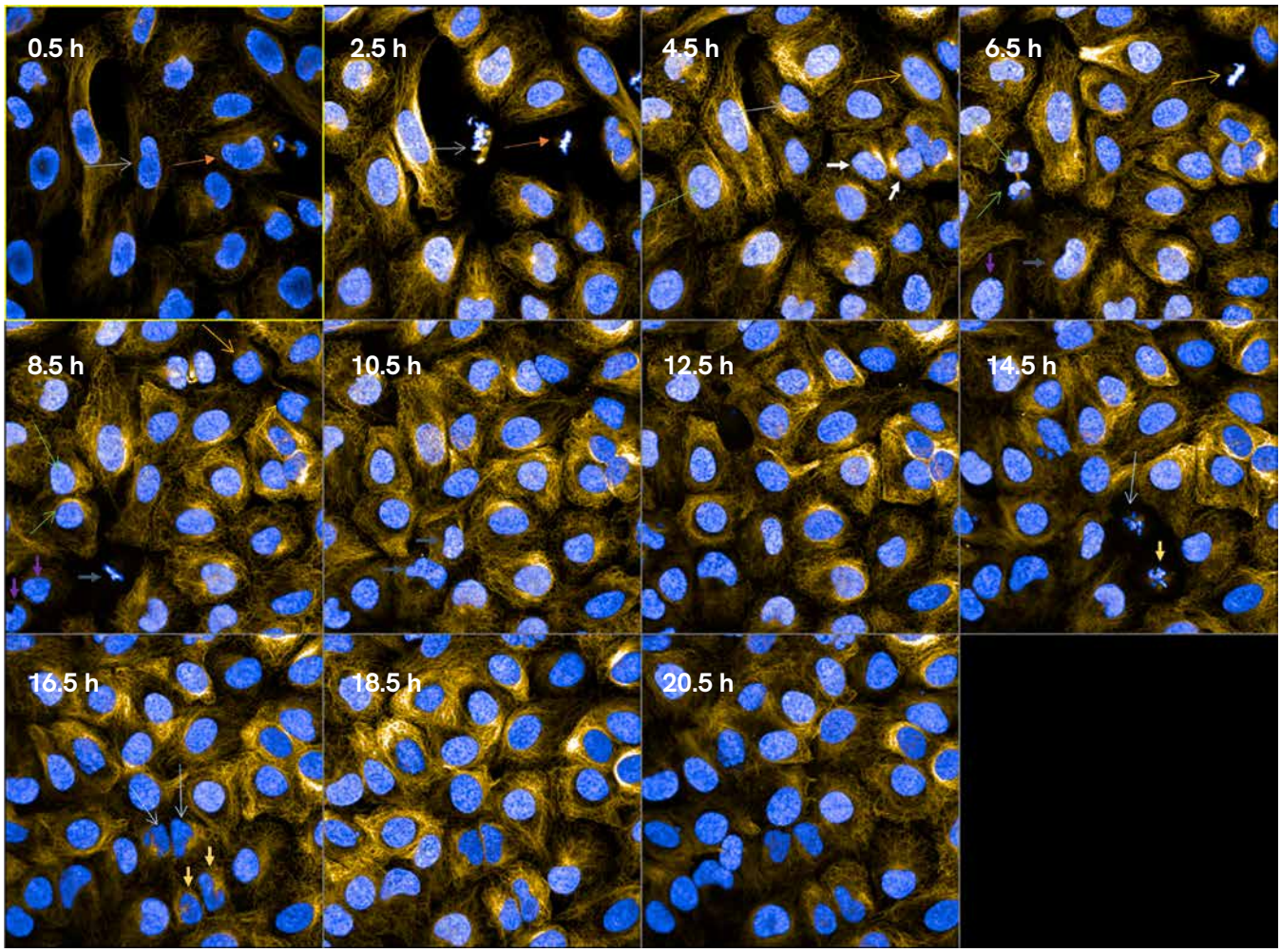


Figure 8: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 4-1,000 nM of PhenoVue Fluor 555 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 and incubated at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system every 2 h for 24 h. 63X water objective confocal. The graph shows the fluorescence quantification for each concentration of PhenoVue Fluor 555 Live Cell Tubulin Stain over time.



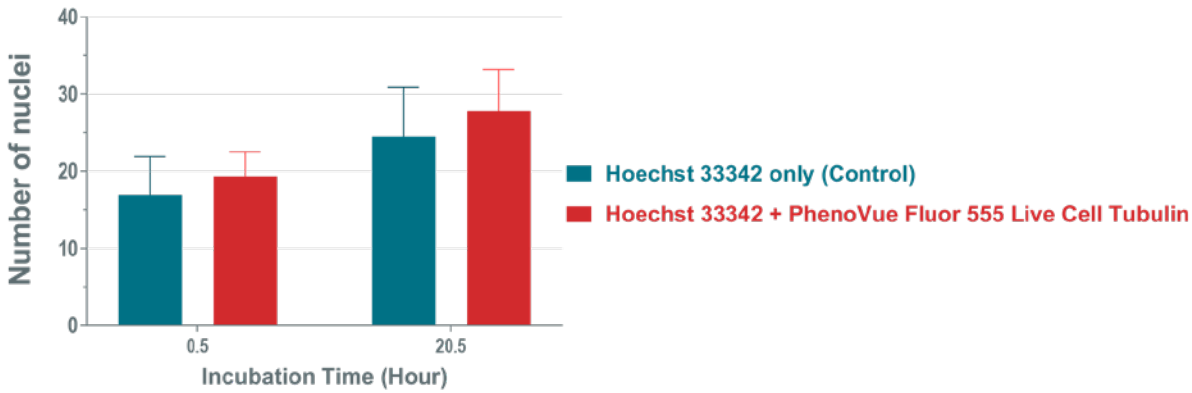


Figure 9: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 4 nM of PhenoVue Fluor 555 Live Cell Tubulin Stain + 0.125 µg/mL PhenoVue Hoechst 33342 and incubated at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system every 2 h for 20 h. 63X water objective confocal. Cell division and proliferation is not impaired by PhenoVue Fluor 555 Live Cell Tubulin Stain, as determined by the quantification of nuclei displayed on the histogram.

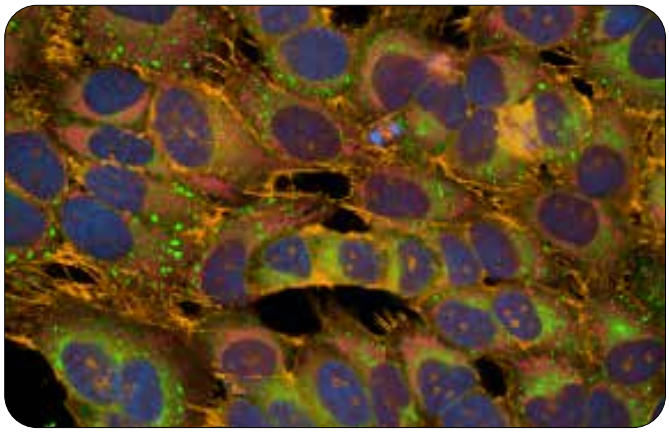


Figure 10: Phenotypic cell painting. HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 Mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated with a Cell Painting mix which includes PhenoVue 512 Nucleic Acid stain (3 µM), PhenoVue Hoechst 33342 nuclear stain (5 µg/mL), PhenoVue Fluor 568 - Phalloidin (33 nM), PhenoVue Fluor 488 - Concanavalin A (100 µg/mL) and PhenoVue Fluor 555 - WGA for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system.

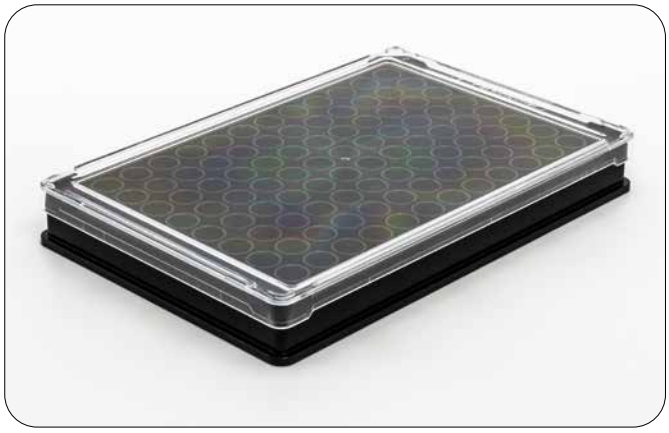


Figure 11: PhenoPlate 96-well microplate

Related products

Opera Phenix® Plus High-Content Screening System

Operetta® CLS™ High-Content Analysis System

Harmony® Imaging and Analysis Software

PhenoPlate high-quality microplates for imaging

PhenoVue Organelle and Cell Compartment Stains

PhenoVue Fluor Secondary Antibody Conjugates

PhenoVue Cell Painting Kits

Revvity
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com