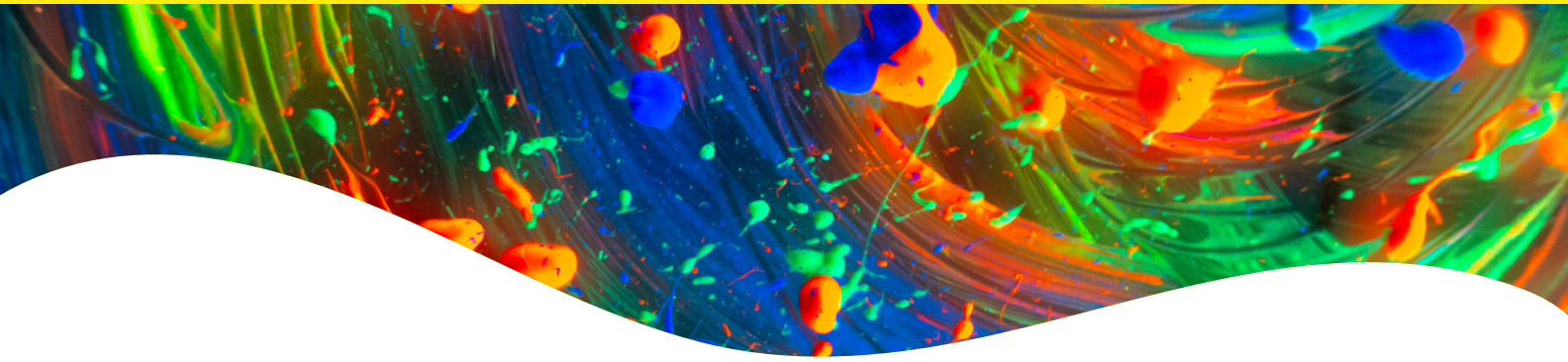
PhenoVue Fluor Live Cell Actin Stain



Overview

PhenoVue™ Fluor 647 live cell actin stain is a no wash, cell permeable fluorogenic dye which specifically bind to actin filaments (F-actin) in live cells. Sensitive, rapid and photostable, PhenoVue Fluor 647 live cell actin stain exhibits far-red emission and can be multiplexed with blue, green and orange colors.

Like other actin stains derived from jasplakinolide, cytotoxicity could 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).

Depending on the cellular model, intracellular retention of PhenoVue Fluor 647 Live Cell Actin 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 647 live cell actin stain – 1x5 nmol	CP22R1	1	1x5 nmol	Liquid (DMSO)	Dry ice
PhenoVue Fluor 647 live cell actin stain – 3x5 nmol	CP22R2	3	3x5 nmol	Liquid (DMSO)	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 30min before taking an aliquot for your experiment. This will increase experiment-to-experiment reproducibility.

Recommended concentration

Product name	Stock concentration	Working concentration range*
PhenoVue Fluor 647 live cell actin stain	100 µM in DMSO	Incubation < 10h: 30 – 100 nM Incubation > 10h: 100 – 300 nM Optimization range: 30 – 300 nM

* Dilutions in cell culture medium.

Equivalent number of microplates

Product name	When Used at Concentration	96-well microplate (100 µL - 300 µL per Well)	384-well microplate (25 µL - 90 µL per Well)	1536-well microplate (4µL - 12µL per Well)
PhenoVue Fluor 647 live cell actin stain – 1x5 nmol	100 nM	1.7 to 5	1.4 to 5	2.7 to 8
PhenoVue Fluor 647 live cell actin stain – 3x5 nmol	100 nM	5 to 15	4 to 15	8 to 24

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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 647 live cell actin stain	650	670	Cy5	140,000

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

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixed-cell staining
PhenoVue Fluor 647 live cell actin stain	Yes	Yes

Protocol

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 647 live cell actin stain is recommended for live cells and compatible on fixed cells.

Live-cell imaging

1. Remove cell culture medium.

2. Incubate with PhenoVue Fluor 647 live cell actin stain in cell culture medium for 30 min at 37 °C, 5% CO_2.

PhenoVue Fluor 647 live cell actin stain:
< 10h incubation: 100 – 300 nM
>10h incubation: 30 – 100 nM
Optimization range: 30 – 300 nM

A nuclear staining dye, such as PhenoVue Hoechst 33342, can be mixed with PhenoVue Fluor live cell actin stain.

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

Recommendations

- We recommend not to remove the cell culture medium containing the PhenoVue Fluor 647 live cell actin stain prior to the time lapse acquisition (no wash experiment). As the dye is fluorogenic, it becomes fluorescent only when bound to F-actin and displays very low fluorescence background when free in medium or cells.
- We recommend no-wash experiments. PhenoVue Fluor 647 live cell actin stain binds F-actin 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 647 live cell actin stain should be optimized depending on the cellular model studied.
- Note that cytotoxicity of staining reagents, such as actin probes, could be observed in long term imaging and is especially accentuated with high concentration as well as high acquisition frequency and power.
- To limit cytotoxic effects (especially for long incubation time), we recommend using PhenoVue Fluor 647 live cell actin stain at low concentration. Usually, concentrations between 30 and 100 nM results 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 100 ng/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 647 live cell actin stain every day.
- Depending on the cellular model, the addition of efflux pump inhibitor, such as probenecid, could improve intracellular retention of PhenoVue Fluor 647 live cell actin stain 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.
- PhenoVue Fluor 647 live cell actin stain is based on Jasplakinolide analog conjugate, which becomes fluorescent when bound to F-actin. Fluorescent probe staining will be impaired if compounds compete and bind to F-actin.
- PhenoVue Fluor 647 live cell actin stain is highly photostable and enables time-lapse experiments with repeated acquisition cycles.

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

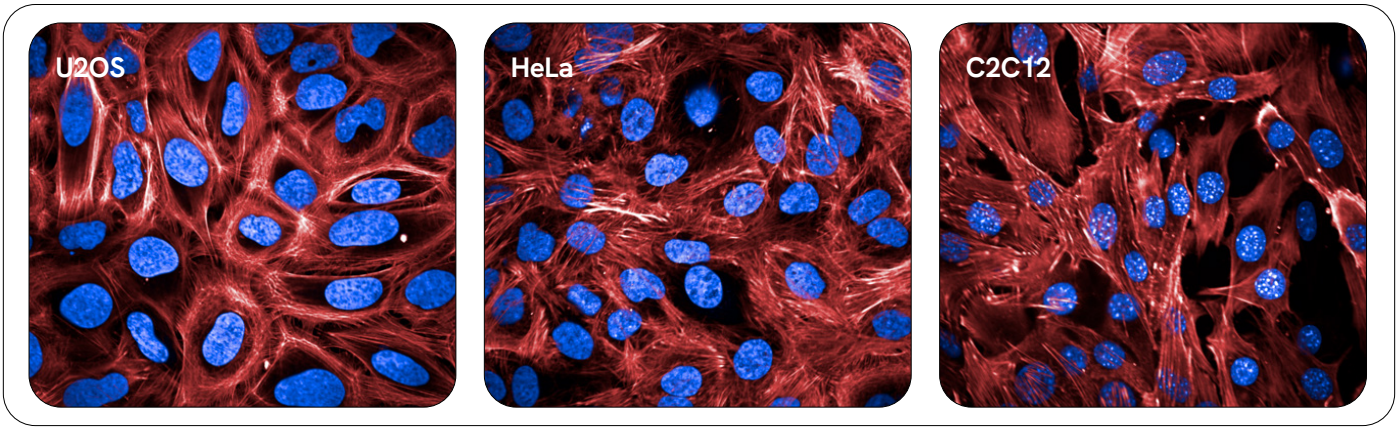

Figure 1: U2OS cells (15,000 cells/well); HeLa cells (40,000 cells/well) and C2C12 cells (20,000 cells/well) were seeded in PhenoPlate™ 96-well microplates and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 100 nM of **PhenoVue Fluor 647 live cell actin stain** + 125 ng/mL **PhenoVue Hoechst 33342** for 2 h 30 min at 37 °C, 5% CO_2. For C2C12 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).

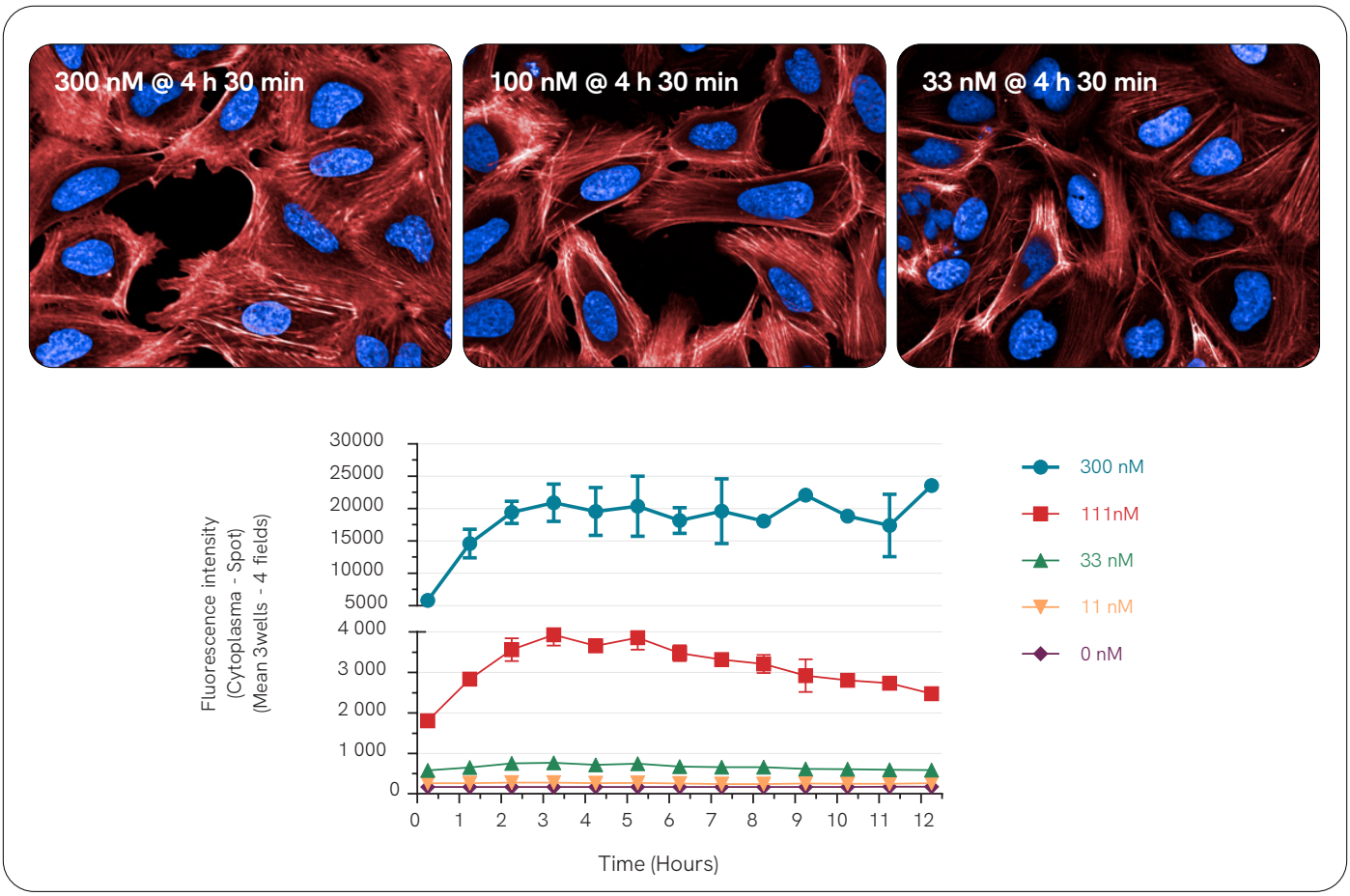

Figure 2: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 11-300 nM of **PhenoVue Fluor 647 live cell acin stain** + 125 ng/mL **PhenoVue Hoechst 33342** and incubated at 37 °C, 5% CO_2. Images were acquired 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 actin stain over time.

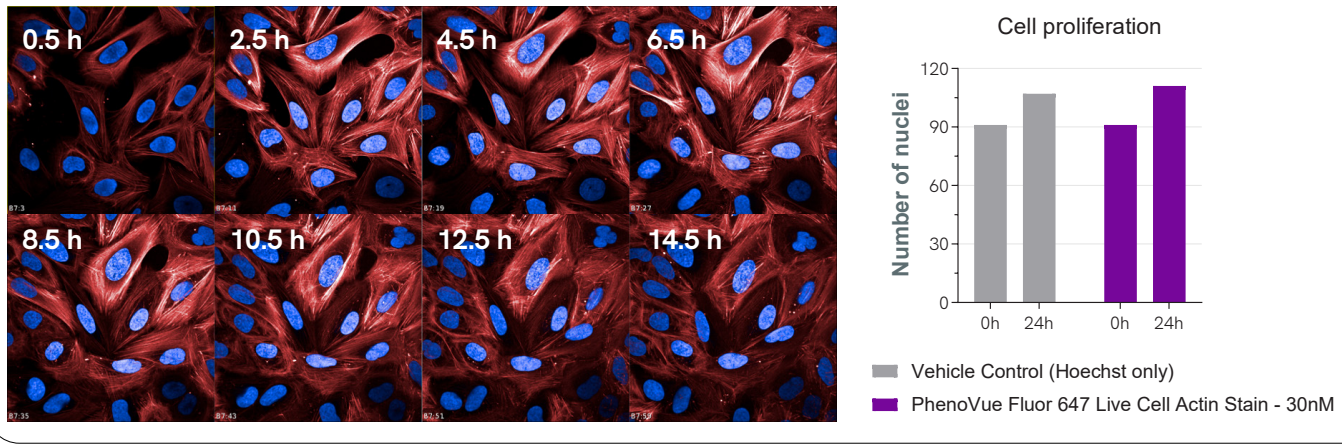

Figure 3: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 37 nM of **PhenoVue Fluor 647 live cell actin stain** + 125 ng/mL **PhenoVue Hoechst 33342** and incubated at 37 °C, 5% CO_2. Images were acquired 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 actin stain, as seen in the images and the number of nuclei displayed in the histogram.

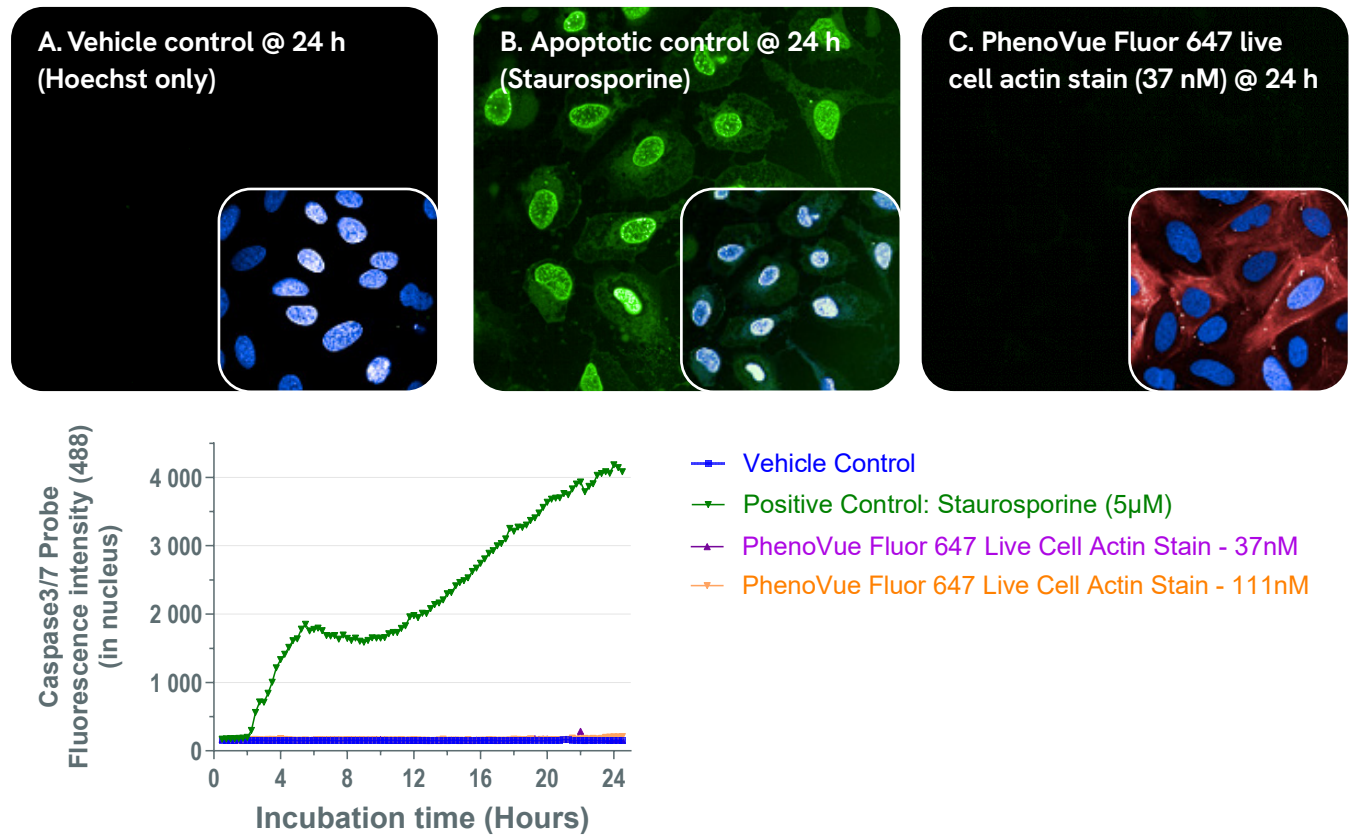

Figure 4: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 2µM **PhenoVue 505 live cell caspase 3/7 activity stain** (apoptotic reporter probe) + 125 ng/mL **PhenoVue Hoechst 33342** +/- 37 or 111 nM of **PhenoVue Fluor 647 live cell actin stain** and incubated at 37 °C, 5% CO_2. Images were acquired on the Opera Phenix Plus high-content screening system every 15 min for 24 h (63X water objective, confocal). Vehicle and apoptotic controls (panel A and B) were incubated without PhenoVue Fluor 647 live cell actin stain. Apoptotic control contains 5 µM staurosporin and shows PhenoVue 505 live cell caspase 3/7 activity stain signal increase in nucleus (panel B and graph) as expected (apoptotic cells). Cells incubated with 37 or 111 nM of PhenoVue Fluor 647 live cell actin stain do not display any apoptotic cells (see panel C and graph, no caspase3/7 activity increase).

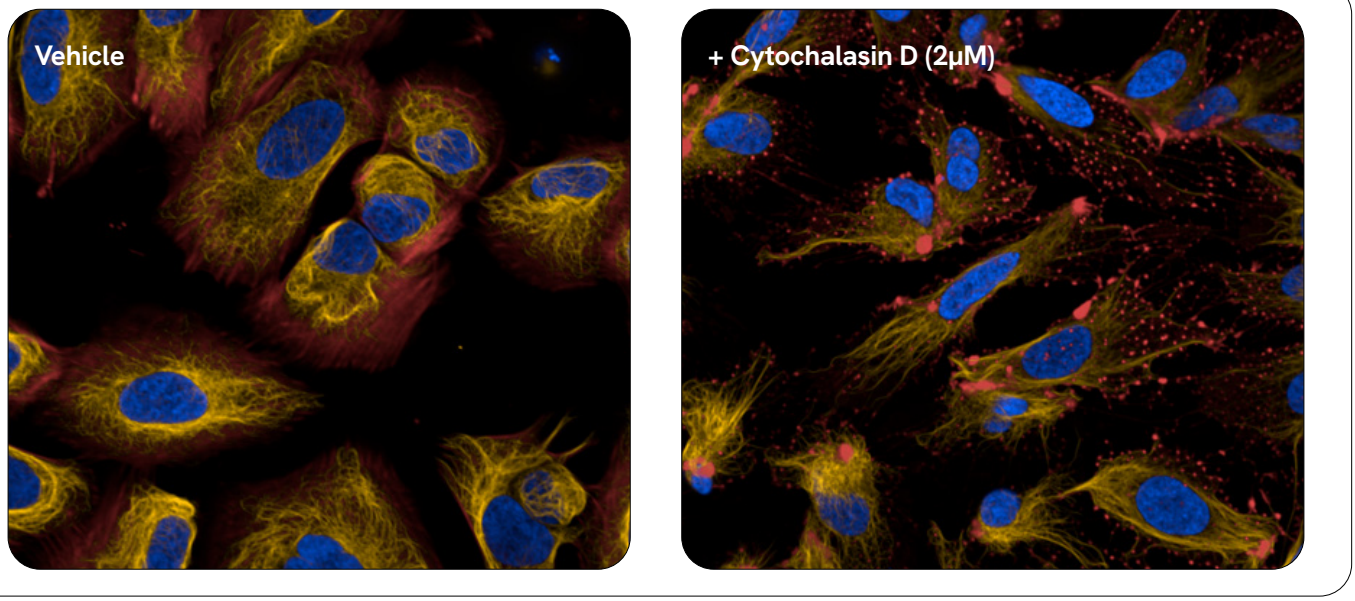

Figure 5: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then stained with 125 ng/mL **PhenoVue Hoechst 33342** (blue) + 100 nM of **PhenoVue Fluor 647 live cell actin stain** (red) + 100nM **PhenoVue Fluor 555 live cell tubulin stain** (orange) and incubated at 37 °C, 5% CO_2 for 30min. Cells were then treated with 2µM cytochalasin D and images were acquired on the Opera Phenix Plus high-content screening system every 5 min for 2.5 h (63X water objective, confocal). Images at 30 min incubation of cytochalasin D are shown. Cytochalasin D, a potent inhibitor of actin polymerization, binds G-actin and turns actin filaments into bundles (Mortensen & Larsson CMLS 2003, Gao et al. BMC Ophthalmology, 2017).

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