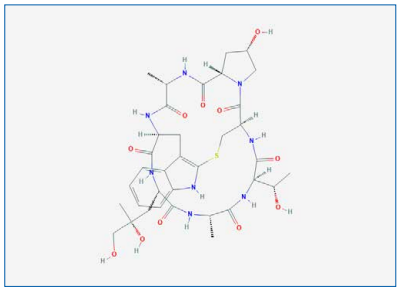
PRODUCT INFORMATION

PhenoVue Fluor - Conjugated Phalloidins

Overview

Phalloidin is a toxin (MW 788.9 g/mol) isolated from the Amanita phalloides mushroom. Phalloidin displays high affinity for F-actin subunits but not for the monomeric G-actin. The interaction between Phalloidin and F-actin stabilizes actin filaments by promoting actin polymerization as well as preventing F-actin dissociation. Therefore, fluorescent Phalloidin conjugates represent a method of choice to label F-actin and cytoskeleton in mammalian cells.



Structure of Phalloidin (Bicyclic heptapeptide with a sulfide bridge). Source: PubChem CID 441542

Product Information

Product Name	Part Number	Number of Vials per Unit	Quantity per Vial	Format	Shipping Conditions
PhenoVue Fluor 488 - Phalloidin	CP24881	1 vial	300 units (10 nmoles, 13 µg)	Desiccated	Dry ice
PhenoVue Fluor 555 - Phalloidin	CP25551	1 vial	300 units (10 nmoles, 13 µg)	Desiccated	Dry ice
PhenoVue Fluor 568 - Phalloidin	CP25681	1 vial	300 units (10 nmoles, 15 µg)	Desiccated	Dry ice
PhenoVue Fluor 594 - Phalloidin	CP25941	1 vial	300 units (10 nmoles, 15 µg)	Desiccated	Dry ice
PhenoVue Fluor 647 - Phalloidin	CP26471	1 vial	300 units (10 nmoles, 13 µg)	Desiccated	Dry ice
PhenoVue Fluor 400LS - Phalloidin	CP24001	1 vial	300 units (10 nmoles, 12 µg)	Desiccated	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 30 min before opening the vials and reconstitution.
- After reconstitution, aliquoted reagents must be stored at -16 °C or below and are stable for 6 months.

PerkinElmer®
For the Better

Recommended Reconstitution

Product Name	Molecular Weight	Recommended Stock Concentration in DMSO	Stock Concentration in Methanol*	Working Concentration Range**
PhenoVue Fluor 488 - Phalloidin	1300 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (87 µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (8.7 µg/mL)	5 – 1000 nM (6.5 ng/mL – 1.3 µg/mL)
PhenoVue Fluor 555 - Phalloidin	1400 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (87µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (8.7 µg/mL)	5 – 1000 nM (6.5 ng/mL – 1.3 µg/mL)
PhenoVue Fluor 568 - Phalloidin	1500 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (100 µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (10 µg/mL)	5 – 1000 nM (7.5 ng/mL – 1.5 µg/mL)
PhenoVue Fluor 594 - Phalloidin	1500 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (100 µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (10 µg/mL)	5 – 1000 nM (7.5 ng/mL – 1.5 µg/mL)
PhenoVue Fluor 647 - Phalloidin	1400 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (87 µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (8.7 µg/mL)	5 – 1000 nM (6.5 ng/mL – 1.3 µg/mL)
PhenoVue Fluor 400LS - Phalloidin	1200 g/mol	Reconstitution using 150 µL DMSO gives a stock concentration of 67 µM (80 µg/mL)	Reconstitution using 1500 µL Methanol gives a stock concentration of 6.7 µM (8 µg/mL)	40 – 400 nM (48 ng/mL – 0.48 µg/mL)

* Due to Methanol evaporation, DMSO reconstitution is preferable.

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

Equivalent Number of Microplates

Product Name	When Used at Recommended Concentration	96-well Plate (100 µL - 300 µL per Well)	384-well Plate (25 µL - 90 µL per Well)	1536-well Plate (4 µL - 12 µL per Well)
PhenoVue Fluor 488 - Phalloidin	165 nM (0.21 µg/mL)	2 to 6	1.5 to 6	3 to 10
PhenoVue Fluor 555 - Phalloidin	55 nM (0.07 µg/mL)	6 to 18	4 to 18	9 to 30
PhenoVue Fluor 568 - Phalloidin	165 nM (0.25 µg/mL)	2 to 6	1.5 to 6	3 to 10
PhenoVue Fluor 594 - Phalloidin	165 nM (0.25 µg/mL)	2 to 6	1.5 to 6	3 to 10
PhenoVue Fluor 647 - Phalloidin	55 nM (0.07 µg/mL)	6 to 18	4 to 18	9 to 30
PhenoVue Fluor 400LS - Phalloidin	165 nM (0.20 µg/mL)	2 to 6	2 to 6	3 to 10

See PerkinElmer's range of high-quality imaging microplates here: www.perkinelmer.com/category/microplates-imaging

Spectral and Photophysical Properties

Product Name	Maximum Excitation Wavelength (nm)	Maximum Emission Wavelength (nm)	Common Filter Set	Quantum Yield (Φ)	Epsilon* (ε in M^{-1} .cm^{-1})	Brightness (Φ x ε)
PhenoVue Fluor 488 - Phalloidin	495	520	FITC	92%	73000	65320
PhenoVue Fluor 555 - Phalloidin	555	570	Cy3	10%	155000	15500
PhenoVue Fluor 568 - Phalloidin	578	603	Texas-Red	69%	88000	60720
PhenoVue Fluor 594 - Phalloidin	590	617	Texas-Red	66%	92000	60720
PhenoVue Fluor 647 - Phalloidin	650	670	Cy5	30%	240000	72000
PhenoVue Fluor 400LS - Phalloidin	395	585	Ex: 375-440 Em: 550-650	nd	26000	nd

* In PBS pH 7.4

Nd: not determined

Live- and Fixed-Cell Compatibility

Product Name	Live-Cell Staining	Fixation/Permeabilization Steps Post Live-Cell Staining	Fixed-Cell Staining
PhenoVue Fluor 488 - Phalloidin	No	No	Yes
PhenoVue Fluor 555 - Phalloidin	No	No	Yes
PhenoVue Fluor 568 - Phalloidin	No	No	Yes
PhenoVue Fluor 594 - Phalloidin	No	No	Yes
PhenoVue Fluor 647 - Phalloidin	No	No	Yes
PhenoVue Fluor 400LS - Phalloidin	No	No	Yes

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.

Phalloidin conjugates are not cell-permeable. Staining requires a prior permeabilization step.

Fixed-Cell Imaging

Rinse briefly in phosphate-buffered saline (PBS) then proceed with cell fixation.

1. **Fixation:** Add ready to use PhenoVue Paraformaldehyde 4% Methanol-Free Solution (PVPFA41) for 10 min at room temperature. Note that paraformaldehyde (PFA) is the most popular fixative reagent.
2. **Washing:** Wash three times with PBS.
3. **Permeabilization:** For PFA fixed cells, add ready to use PhenoVue Permeabilization 0.5% Triton X-100 Solution (PVPERM051) for 10-15 min at room temperature (for membrane-associated antigens, 100 µM digitonin or 0.5% saponin are preferred). Triton X-100 is the most popular detergent for improving the penetration of antibodies. However, it may be not appropriate for some imaging applications since it can destroy membranes.
4. **Washing:** Wash three times with PBS for 5 min.
5. **Staining:** Incubate with 50-200 nM PhenoVue Fluor Phalloidin for 30-45 min at RT*.
6. **Washing:** Wash three times with PBS for 5 min.
7. **Optional:** Incubate with 0.1-2 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.
8. **Washing:** Wash once with PBS for 5 min.
9. Acquire images on an imaging device.

* See recommended concentrations in the table "Equivalent Number of Microplates"

Tips

- For the reconstitution step and stock solution preparation, avoid methanol and other alcohol-based or aqueous solvents. It is preferable to use anhydrous DMSO which preserves the integrity of actin filaments, enabling brighter staining intensity.
- For the fixation step, avoid methanol-based methods. It is preferable to use methanol-free formaldehyde since methanol can disrupt actin.
- Phalloidin conjugates are not cell permeable. Therefore, staining requires prior permeabilization step.
- Please note that PhenoVue Dye Diluent A (recommended for conjugate dilution) contains 1% BSA which might help reducing background signal while avoiding blocking step prior staining.

Special Recommendations for PhenoVue Fluor 400LS - Phalloidin in a 5-Plex Experiment.

PhenoVue Fluor 400LS - Phalloidin is a long Stokes shift dye which allows multiplexing of up to 5 colors. To obtain a high fluorescent signal, please note the following acquisition settings:

- **Excitation of PhenoVue Fluor 400LS between 360 and 415 nm (e.g. Opera Phenix®/Plus with 405 nm or Operetta® CLS™ with 405 or 365 nm excitation):**
  - Reduce the concentration of Hoechst 33342 (or DAPI) to limit its crosstalk to the 570-630 nm detection band. A Hoechst (or DAPI) concentration of 20 - 80 ng/mL (incubated for 30-60 min) typically gives good nuclear staining while significantly reducing the crosstalk.
- **Excitation of PhenoVue Fluor 400LS with greater than 415 nm (e.g. Operetta CLS with 440 nm excitation):**
  - When used together with PhenoVue Fluor 488 conjugates, use a 600-640 nm emission band for PhenoVue Fluor 400LS to limit the crosstalk of PhenoVue Fluor 488.
- **For simultaneous acquisition (e.g. Opera Phenix/Plus):**
  - Separate Hoechst 33342 (Ex: 405/425 nm, Em: 435-480 nm) and PhenoVue Fluor 555 / 568 (Ex: 561 nm; Em: 570-630 nm) channels. 405 or 425 nm excitation of PhenoVue Fluor 400LS Phalloidin may result in an emission in the 570-630 nm detection band.

HCS Instruments		PhenoVue Hoechst 33342	PhenoVue Fluor 400LS Phalloidin	PhenoVue Fluor 488	PhenoVue Fluor 555 or Fluor 568	PhenoVue Fluor 647
Opera Phenix Plus 5 lasers	Excitation laser	375	425	488	561	640
	Emission filter	435-480	570-630	500-550	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation laser	405	405	488	561	640
	Emission filter	435-480	570-630	500-550	570-630	650-760
Operetta CLS 8 LED - 1600	Excitation LED (filter)	370 (355-385)	405 (390-420)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	570-650	500-550	570-650	655-760
Operetta CLS 8 LED - 1601	Excitation LED (filter)	370 (355-385)	440 (435-460)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	600-640 or 570-650	500-550	570-650	655-760
Operetta CLS 4 LED	Excitation LED (filter)	370 (355-385)	370 (355-385)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	570-650	500-550	570-650	655-760

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
- Flow Cytometry

Validation Data

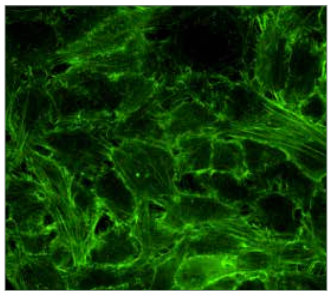


*Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and stained with 165 nM of **PhenoVue Fluor 488 - Phalloidin** for 45 min at RT. Images were acquired on the Operetta CLS™ high-content analysis system.*

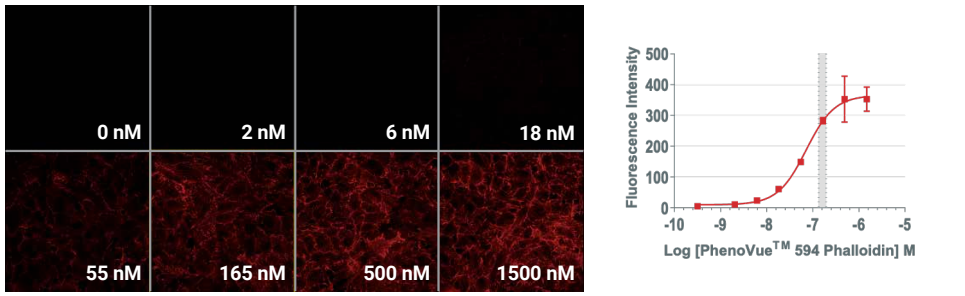


*Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and stained with increasing concentrations of **PhenoVue Fluor 594 - Phalloidin** for 45 min at RT. Images were acquired on the Operetta CLS high-content analysis system.*

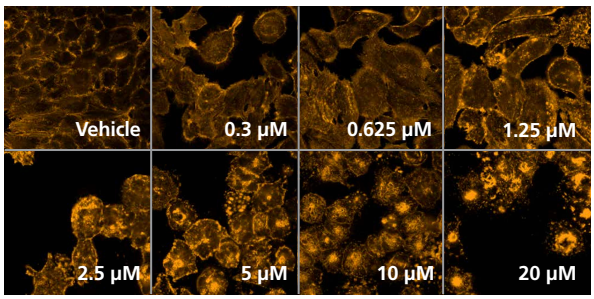


*Figure 3: HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were treated with increasing concentrations of Cytochalasin D for 48h. Cytochalasin D binds to G-actin and induces actin depolymerization (Mortensen & Larsson 2003, Gao et al. 2017, Kim et al. 2012). Cells were fixed then permeabilized and stained with 33 nM of **PhenoVue Fluor 568 - Phalloidin** for 30 min at RT. Images were acquired on an Operetta CLS high-content analysis system.*

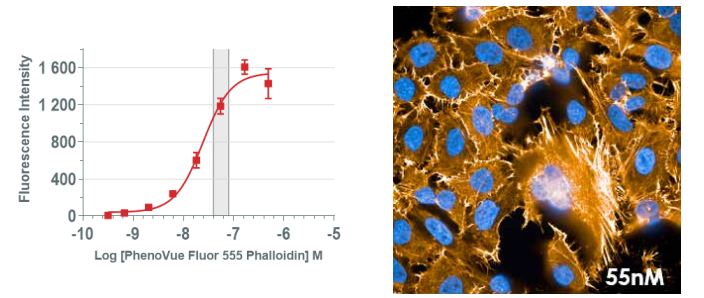


*Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and stained with increasing concentrations (1-500nM) of **PhenoVue Fluor 555 - Phalloidin** for 45 min at RT. Images were acquired on the Operetta CLS high-content analysis system.*

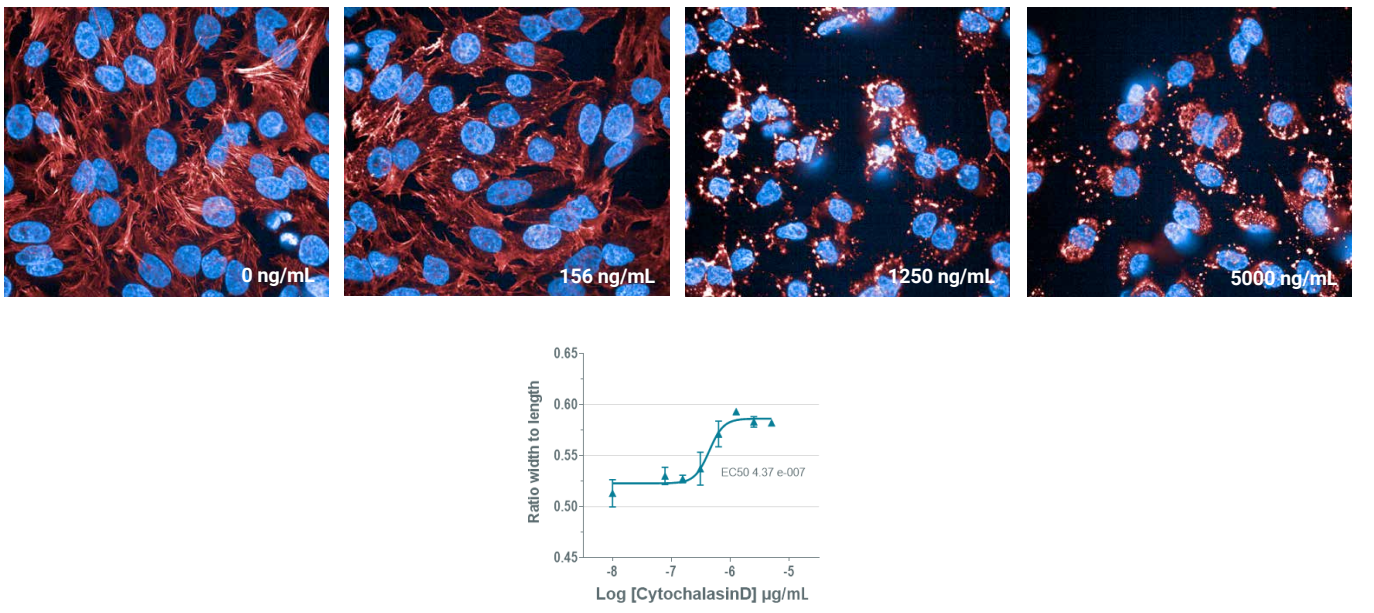


*Figure 5: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were treated with increasing concentrations of Cytochalasin D for 1h. Cytochalasin D binds to G-actin and induces actin depolymerization (Mortensen & Larsson 2003, Gao et al. 2017, Kim et al. 2012). Cells were fixed then permeabilized and stained with 55 nM of **PhenoVue Fluor 647 - Phalloidin** for 30 min at RT. Images were acquired on an Operetta CLS high-content analysis system.*

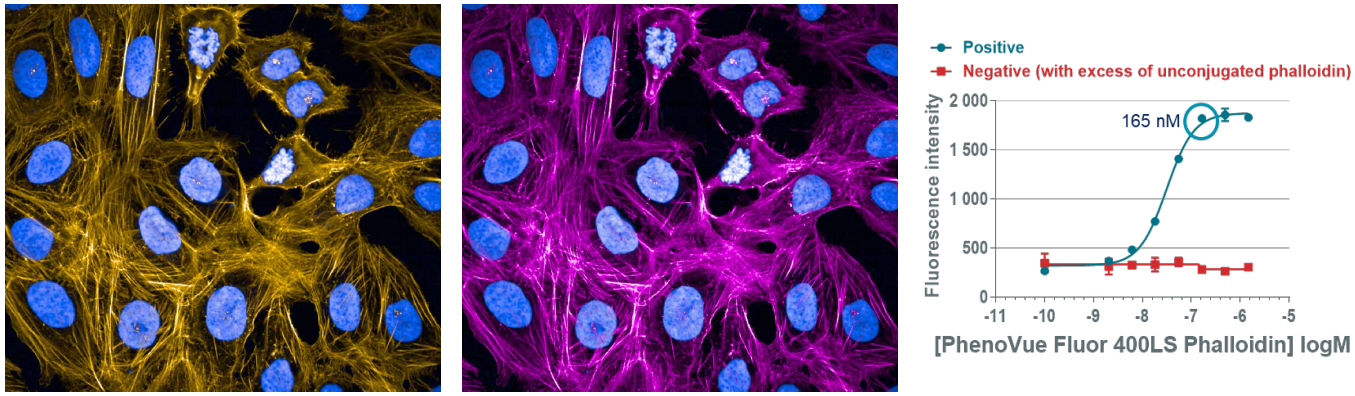


*Figure 6: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed (PhenoVue Paraformaldehyde 4%) then permeabilized (PhenoVue Permeabilization 0.5% Triton X-100 Solution) and stained with increasing concentrations (2-1500 nM) of **PhenoVue Fluor 400LS – Phalloidin** (displayed in orange or fuchsia pseudo colors) + PhenoVue Hoechst 33342 (2 µg/mL) (diluted in PhenoVue Dye Diluent A) for 45 min at RT. Background staining signal (negative condition) was monitored with preincubation of an excess of unconjugated phalloidin for 30 min at RT. Images were acquired on the Opera Phenix Plus (PhenoVue Fluor 400LS - Ex: 425 nm laser ; Em: 570-630 nm / PhenoVue Hoechst 33342 – Ex: 375 nm laser ; Em: 435-480 nm) high-content analysis system with 63X water objective.*

Acquisition settings

		PhenoVue Hoechst 33342	PhenoVue Fluor 400LS Phalloidin	PhenoVue Fluor 488 Concanavalin A	PhenoVue Fluor 555 WGA	PhenoVue 641 Mitochondrial stain
Opera Phenix Plus 5 lasers	Excitation	375	425	488	561	640
	Emission	435-480	570-630	500-550	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation	405	405	488	561	640
	Emission	435-480	570-630	500-550	570-630	650-760
Operetta CLS 8 LED - 1600	Excitation	370 (355-385)	405 (390-420)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission	430-500	570-650	500-550	570-650	655-760
Operetta CLS 8 LED - 1601	Excitation	370 (355-385)	440 (435-460)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission	430-500	600-640 or 570-650	500-550	570-650	655-760
Operetta CLS 4 LED	Excitation	370 (355-385)	370 (355-385)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission	430-500	570-650	500-550	570-650	655-760

Figure 7: 5-Plex experiment acquired on different Opera Phenix Plus and Operetta CLS configurations. U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were first stained with PhenoVue 641 Mitochondrial stain (500nM in PhenoVue Dye diluent A) for 30 min at 37 °C + 5% CO_2. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT) then permeabilized (PhenoVue Permeabilization 0.1% Triton X-100 Solution – 15 min at RT) and stained with a mix of PhenoVue Hoechst 33342 nuclear stain (70ng/mL) + PhenoVue Fluor 400LS Phalloidin (165 nM) + PhenoVue Fluor 488-ConcanavalinA (5 µg/mL) + PhenoVue Fluor 555-WGA (1.5 µg/mL) in PhenoVue dye Diluent A for 30 min at RT. Images were acquired on the Opera Phenix Plus (5 lasers and 4 lasers) and Operetta CLS (8 LED, 1600) high-content analysis system with the 63X water objective. The table describes the recommended acquisition settings for the five different configurations of Opera Phenix Plus and Operetta CLS.

Related Products

Opera Phenix Plus High-Content Screening System
www.perkinelmer.com/operaphenixplus

Operetta CLS High-Content Analysis System
www.perkinelmer.com/operettaCLS

Harmony® Imaging and Analysis Software
www.perkinelmer.com/harmony

PhenoPlate high-quality microplates for imaging
www.perkinelmer.com/PhenoPlates

PhenoVue Cell Painting Kits
www.perkinelmer.com/PhenoVue

PhenoVue Fluor Secondary Antibody Conjugates
www.perkinelmer.com/PhenoVue

PhenoVue Organelle and Cell Compartment Stains
www.perkinelmer.com/PhenoVue

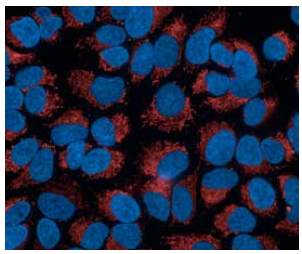


*Figure 8: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Live cells were stained with 150 nM of **PhenoVue Fluor 641 Mitochondrial stain** for 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS high-content analysis system.*

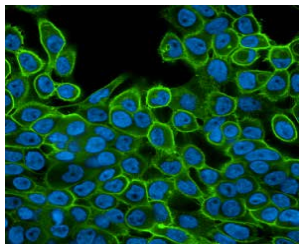


*Figure 9: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of **PhenoVue Fluor 488 Goat Anti-Mouse IgG (H+L) Cross-adsorbed** for 1 hour at RT. Nuclei were stained with 5 µg/mL PhenoVue Hoechst 33342 nuclear stain. Images were acquired on the Operetta CLS high-content analysis system.*

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

1039207 (775539) PKI