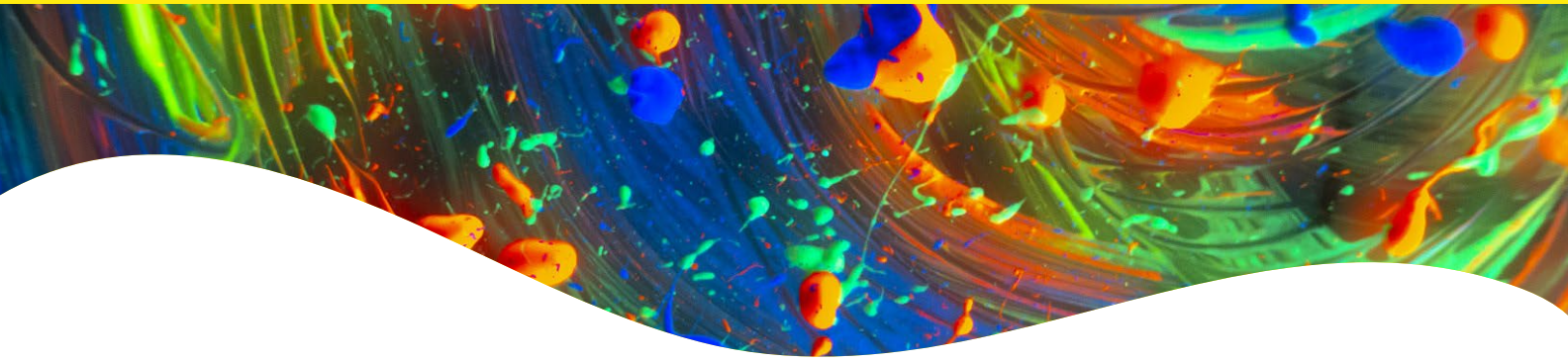
PhenoVue Cell Painting Kit (for 10 x 384-well Plates)



Overview

Cell painting is a powerful phenotypic high-content screening approach which combines cell and computational biology to unravel cells' responses when subjected to perturbagens. Cells are "painted" by labelling different cellular compartments with different fluorescent bioprobes to quantitatively profile multiple phenotypic parameters in order to better understand the effects of chemical compounds, drugs, genes, or other test articles. Cell compartments and organelles are simultaneously tagged with six fluorescent probes, followed by acquisition and analysis of images. The six probes target specific cell compartments to determine protein expression or signaling pathways, to identify organelles and their function, or identify whole-cell morphology.

The PhenoVue™ cell painting kit comprises validated, pre-optimized fluorescent bioprobes to streamline your workflow, saving time and costs.

Product information

Product name	Part no.	Number of vials per kit	Shipping conditions
PhenoVue cell painting kit - 10 x 384 wells	PING12	8	Dry ice

Kit contents	Format	Quantity	Storage
PhenoVue Fluor 555 - WGA	Lyophilized	1 vial (0.2 mg)	2-8 °C or below. Protect from light.
PhenoVue Fluor 488 - Concanavalin A	Lyophilized	2 vials (6 mg per vial)	2-8 °C or below. Protect from light.
PhenoVue Fluor 568 - Phalloidin	Dessicated	1 vial (4 nmol)	-16 °C or below. Protect from light.
PhenoVue 641 mitochondrial stain	Dessicated	1 vial (50 µg)	-16 °C or below. Protect from light.
PhenoVue Hoechst 33342 nuclear stain	Solution in H_2O	1 vial (700 µg, 700 µL)	2-8 °C or below. Protect from light.
PhenoVue 512 nucleic acid stain	Solution in DMSO	1 vial (400 nmol, 80 µL)	-16 °C or below. Protect from light.
PhenoVue dye diluent A (5x)	Liquid	1 vial (80 mL)	2-8 °C or below.

Storage

For convenience, store the kit at ≤ -16 °C. However, each reagent can be stored separately between ≤ -16 °C to 2-8 °C, as indicated in the table above. Avoid repeated freeze / thaw cycles. After reconstitution, aliquoted reagents must be stored at -16 °C or below.

After thawing, the PhenoVue dye diluent A (5x) may contain some aggregates which will not impair the product and image quality. If aggregate removal is preferred, the PhenoVue dye diluent A (5x) can be filtered (0.22 µm filter) prior to dilution. The resulting PhenoVue dye diluent A 1X must be stored at 2-8 °C for no more than 2 days.

Stability

The stability of the kit is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.

Other materials and reagents not provided

Reagent	Use
HBSS buffer	Washing buffer & diluent for PFA & Triton X-100
Paraformaldehyde (PFA), methanol free	Fixation buffer
Triton X-100	Permeabilization buffer
DMSO	Reconstitution of PhenoVue 641 mitochondrial stain and PhenoVue Fluor 568 - Phalloidin
Methanol (optional)	Reconstitution of PhenoVue Fluor 568 - Phalloidin (optional)
PhenoPlate™ 384-well microplates*	Cell plating, stimulation, staining and imaging
Aluminium single-tab foil	Plate sealing to protect fluorescent probes from light

*This protocol can be adapted using PhenoPlate 96-well microplates. See Protocol Section for details. View our full range of high-quality imaging microplates at Revvity.com

Reagent reconstitution and preparation of staining solutions

1. **Prepare stock solutions of PhenoVue dye diluent A and Stains as described in the table below.**
2. **Prepare two Staining Solutions:**

Staining Solution 1: Comprises PhenoVue 641 Mitochondrial Stain and is intended to be used for mitochondrial staining of live cells.

Staining Solution 2: Cell painting mix that is intended to be used on fixed and permeabilized cells and includes:

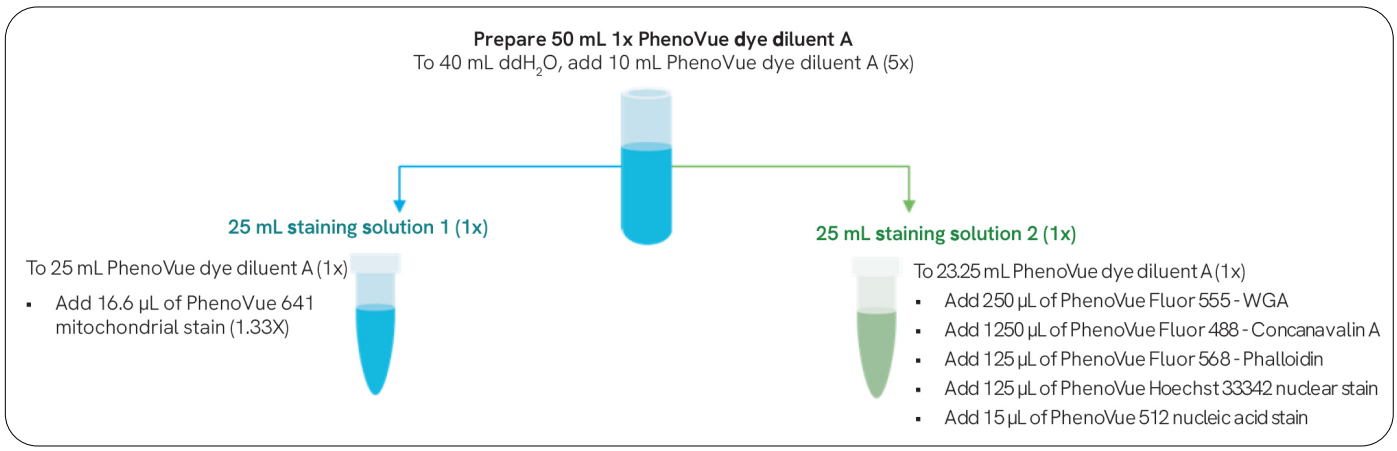
- PhenoVue Fluor 555 - WGA
- PhenoVue Fluor 488 - Concanavalin A
- PhenoVue Fluor 568 - Phalloidin
- PhenoVue Hoechst 33342 nuclear stain
- PhenoVue 512 nucleic acid stain

Note: Protect stock and staining solutions from light.

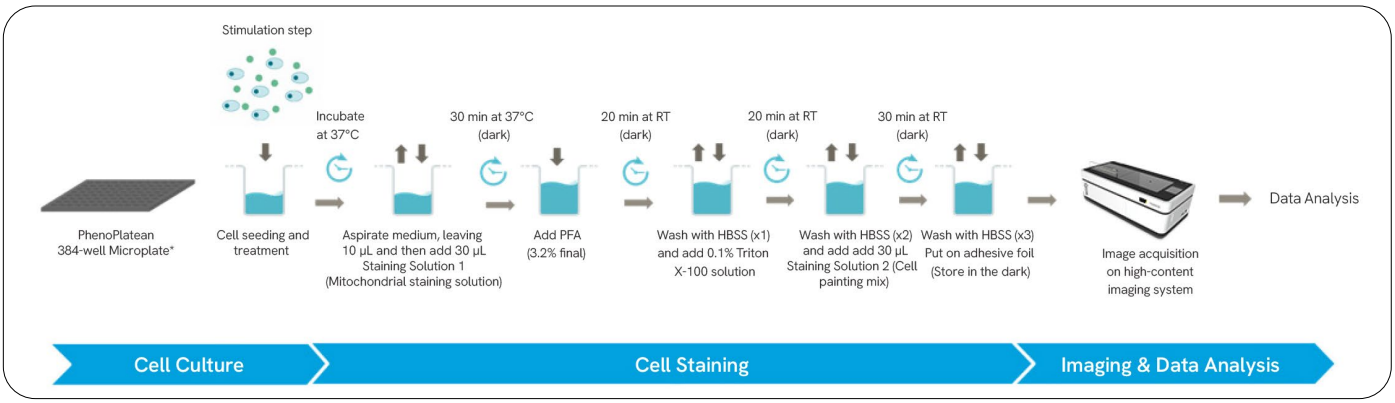
	Reagent name	1. Reconstitution/preparation of stock solution	2. Preparation of staining solutions	Final concentration of reagents per well
	PhenoVue dye diluent A (5x)	Dilute 5 times in distilled H_2O to give a 1x ready to use buffer.	Ready to use for dilution of other reagents.	HBSS + 1 % BSA (1x)
Staining Solution 1	PhenoVue 641 mitochondrial stain	Reconstitute with 90 µL DMSO to give a 1 mM (2000x) stock solution.	Dilute stock solution 2000 times in PhenoVue dye diluent A (1x) or cell culture medium to give a 500 nM staining solution. IMPORTANT: always consider the total volume per well, e.g. 10 µL of cells + 30 µL of staining solution 1 prepared at 1.33x (666.6 nM).	500 nM (272 ng/mL)
Staining Solution 2	PhenoVue Fluor 555 - WGA	Reconstitute with 1.3 mL dH_2O to give a 0.15 mg/mL (100x) stock solution.	Dilute stock solution 100 times in PhenoVue dye diluent A (1x) to give a 1.5 µg/mL staining solution.	43.7 nM (1.5 µg/mL)
	PhenoVue Fluor 488 - Concanavalin A	Reconstitute (each vial) with 3 mL dH_2O to give a 2 mg/mL (20x) stock solution.	Dilute stock solution 20 times in PhenoVue dye diluent A (1x) to give a 100 µg/mL staining solution.	960 nM (100 µg/mL)
	PhenoVue Fluor 568 - Phalloidin	Reconstitute with 600 µL DMSO to give a 6.6 µM (200x) stock solution, or Reconstitute with 600 µL Methanol to give a 6.6 µM (200x) stock solution.	Dilute stock solution 200 times in PhenoVue dye diluent A (1x) to give a 33 nM staining solution.	33 nM (48 ng/mL)
	PhenoVue Hoechst 33342 nuclear stain	Ready to use stock solution at 1 mg/mL (200x).	Dilute stock solution 200 times in PhenoVue dye diluent A (1x) to give a 5 µg/mL staining solution.	8.12 µM (5 µg/mL)
	PhenoVue 512 nucleic acid stain	Ready to use stock solution at 5 mM (1666x).	Dilute stock solution 1666 times in PhenoVue dye diluent A (1x) to give a 3 µM staining solution.	3 µM (1.43 µg/mL)

Example preparation of staining solutions

The following example describes the preparation of 25 mL Staining Solution 1 and 25 mL Staining Solution 2, sufficient for approximately 2 x 384-well plates



Experimental workflow



*This protocol can be adapted using PhenoPlate 96-well microplates. See Protocol Section for details.

Protocols

This protocol has been adapted from Bray et al.*

1. **Dispense** 40 µL of cells per well into PhenoPlate 384-well microplates** and incubate at 37 °C, 5% CO_2 overnight. Typical cell seeding density for this application is in the range of 400 - 2000 cells/well, depending on cell type and duration of compound treatment.
2. **Add** compounds and incubate at 37 °C, 5% CO_2 typically for 24 to 48h.
3. **Aspirate** cell culture medium from each well, leaving 10 µL to minimize disturbance to the live cells from pipetting and media turbulence.
4. **Add** 30 µL of staining solution 1.
5. **Centrifuge** the plate (500 g at RT for 1 min).
6. **Incubate** for 30 min in the dark at 37 °C.

Perform the following steps with **no pauses:**

7. **Add** 10 µL of 16% (wt/vol) methanol-free PFA (3.2% final concentration) (vol/vol).
8. **Centrifuge** the plate (500 g at RT for 1 min).
9. **Incubate** at RT for 20 min in the dark.
10. **Wash** once with 70 µL of 1x HBSS.
11. **Discard** HBSS.
12. **Add** 30 µL of HBSS - 0.1% (vol/vol) Triton X-100.
13. **Centrifuge** the plate (500 g at RT for 1 min).
14. **Incubate** at RT for 10 - 20 min in the dark.
15. **Wash** twice with 70 µL of 1x HBSS.
16. **Discard** HBSS.
17. **Add** 30 µL of staining solution 2.
18. **Centrifuge** the plate (500 g at RT for 1 min).
19. **Incubate** at RT for 30 min in the dark.
20. **Wash** three times with 70 µL of 1x HBSS.
21. **Do not discard** the final 70 µL HBSS.
22. **Seal the plates** with adhesive foil and store them at 4 °C in the dark until ready to image.
23. **Automated image acquisition**:
    - Place the microplates in the Opera Phenix® Plus High-Content Screening System or other automated imaging microscopy system.
    - Set up the microscope acquisition settings as described in Bray et al.
    - Start the automated imaging sequence according to the microscope manufacturer's instructions.
24. **Image Analysis:** Refer to Bray et al.*, for detailed data reduction protocol.

*Bray, MA., Singh, S., Han, H. et al. Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes. Nat Protoc 11, 1757–1774 (2016).

**PhenoPlate 96-well microplates may also be used. Adjust the cell density accordingly and increase the corresponding volumes by 2.5-fold.

Validation data

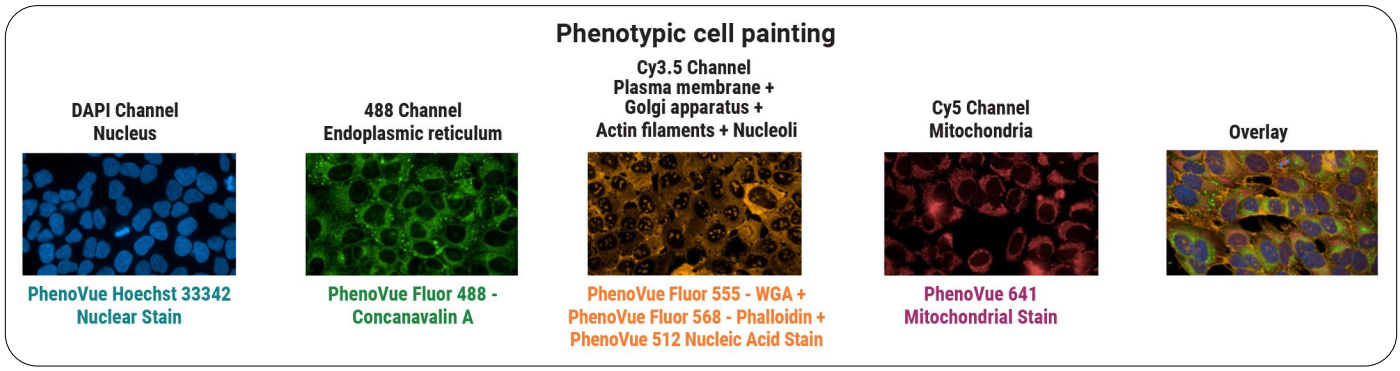


Figure 1: HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 Mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated with the Cell Painting mix 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 (1.5 µg/mL) for 30 min at RT. Images were acquired on the Operetta CLS™ high-content analysis system (63x water objective, confocal mode).

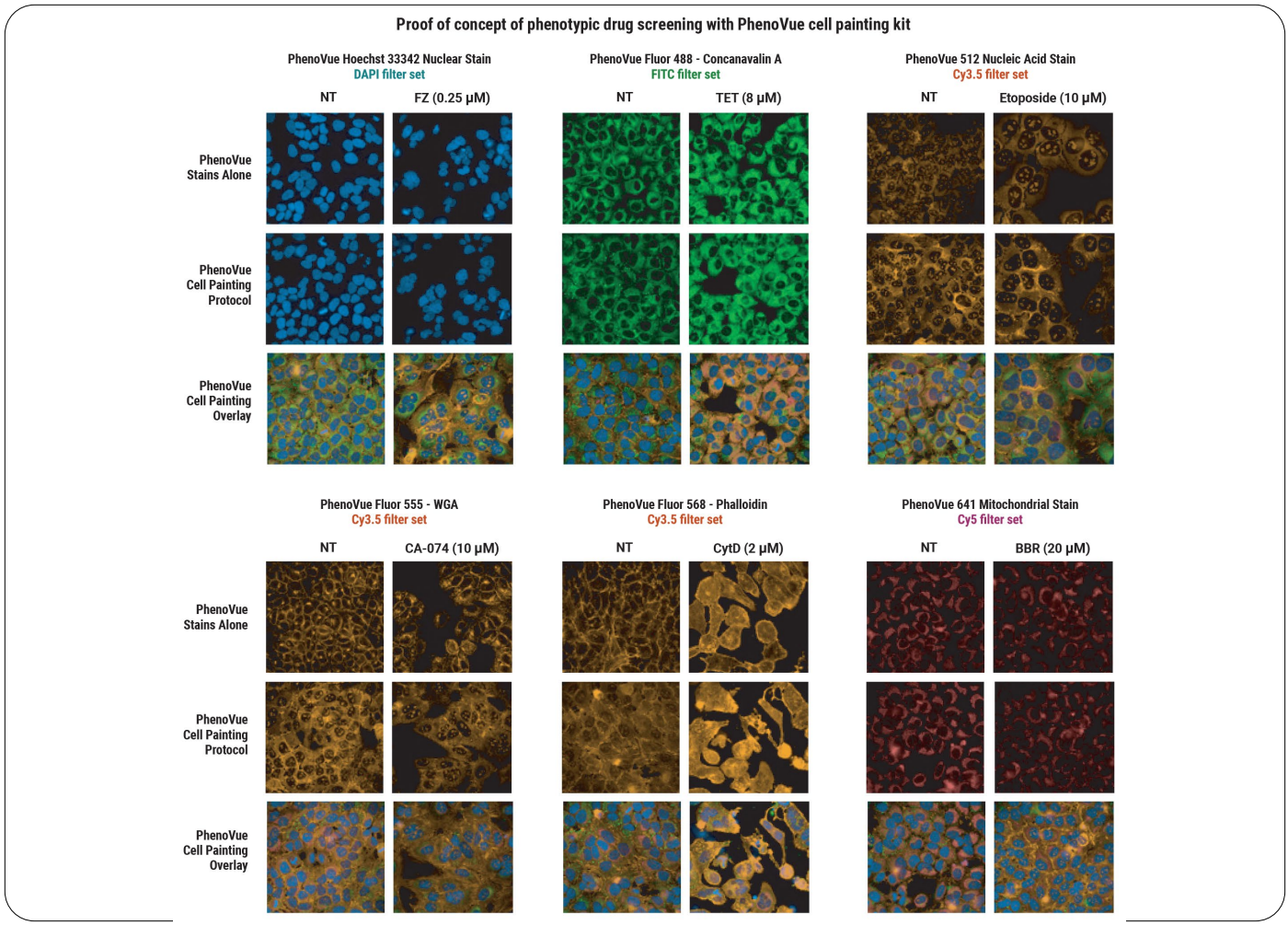


Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were then untreated or treated for 48h with the following compounds prior to applying PhenoVue reagents alone or the cell painting protocol:

- Fenbendazole (FZ, Sigma #35032) binds to tubulin alpha chain, inhibiting microtubule polymerization. FZ induces mitotic spindle disruption and eventually leads to mitotic catastrophe which can be visualized as giant, multinucleated cells[1].
- Tetrandrine (TET, SantaCruz Biotech #sc-201492) which is a potent inhibitor of P-glycoprotein-mediated multidrug resistance and has been shown to increase endoplasmic reticulum abundance[2,3].
- Etoposide (Tocris, #1226) is a topoisomerase II inhibitor which induces double stranded DNA breaks and G2 cell cycle arrest resulting in increase of cell, nucleus and nucleoli enlargement[2,3].
- CA-074 methyl ester (CA-074, SantaCruz Biotech #sc-214647) is a Cathepsin B Inhibitor shown to increase Golgi apparatus abundance.
- Cytochalasin D (CytD, Sigma #C8273) binds to G-actin and induces depolymerization of actin filaments[4,5].
- Berberine Chloride (BBR, Sigma #PHR1502) modulates lipid metabolism and decreases cholesterol blood level[6]. BBR has been shown to modulate mitochondria localization and compactness.

Images were acquired on the Operetta CLS high-content analysis system using filter sets as indicated on each panel (63x water objective, confocal mode). Note that each compound displays the expected phenotype, demonstrating the performance of the PhenoVue cell painting kit.

References: [1]Dogra et al. *Sci Rep 2018, [2]Gustafsdottir* et al. *PLoS One 2013, [3]Nyffeler* et al. *Toxicology and Applied Pharmacology 2020, [4]Mortensen & Larsson. 2003, [5] Gao et al. 2017, [6]Wang* et al. *Cholesterol 2018*

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