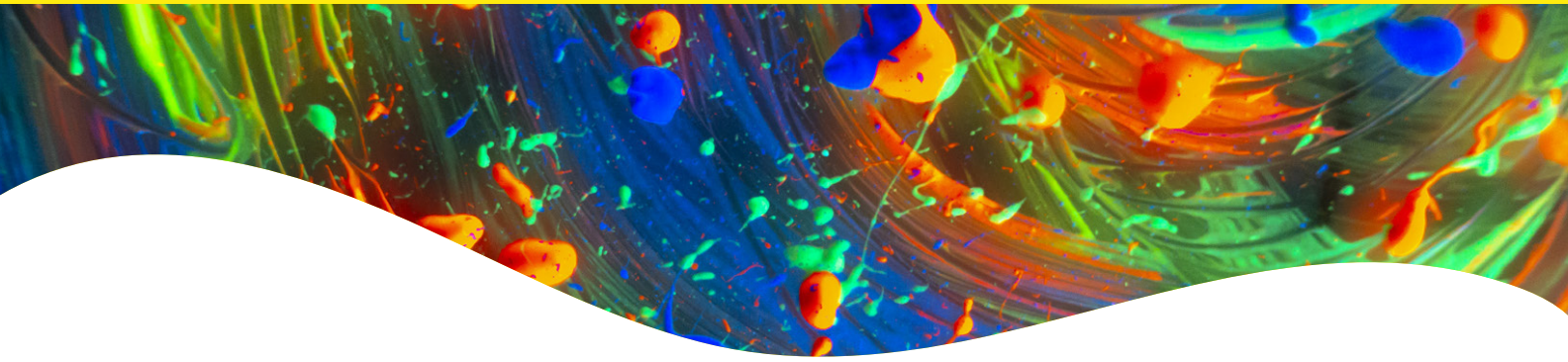
PhenoVue Cell Painting JUMP Kit (for 1 x 384-well Plate)



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 JUMP kit comprises validated, pre-optimized fluorescent bioprobes, according to the JUMP consortium protocol v3 to streamline your workflow, saving time and costs.

Product information

Product name	Part no.	Number of vials per kit	Shipping conditions
PhenoVue Cell Painting JUMP Kit - 1 x 384 wells	PING21	7	Dry ice

Kit contents	Format	Quantity	Storage
PhenoVue Fluor 555 - WGA	Lyophilized	1 vial (0.02 mg)	2-8 °C or below. Protect from light.
PhenoVue Fluor 488 - Concanavalin A	Lyophilized	1 vial (1.2 mg)	2-8 °C or below. Protect from light.
PhenoVue Fluor 568 - Phalloidin	Dessicated	1 vial (0.4 nmol)	-16 °C or below. Protect from light.
PhenoVue 641 Mitochondrial Stain	Dessicated	1 vial (22 µg)	-16 °C or below. Protect from light.
PhenoVue Hoechst 33342 Nuclear Stain	Solution in H_2O	1 vial (70 µg, 70 µL)	2-8 °C or below. Protect from light.
PhenoVue 512 Nucleic Acid Stain	Solution in DMSO	1 vial (100 nmol, 20 µL)	-16 °C or below. Protect from light.
PhenoVue Dye Diluent A (5x)	Liquid	1 vial (8 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
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:

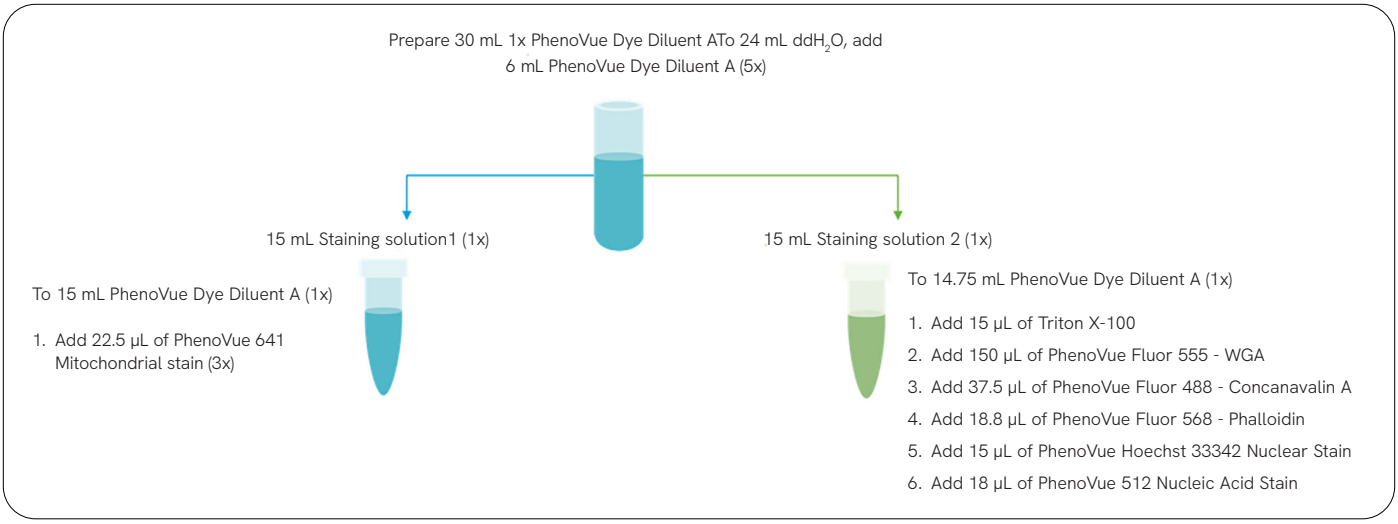
- Triton X-100 (0.1% final)
- PhenoVue Fluor 555 - WGA
- PhenoVue Fluor 488 - Concanavalin A
- PhenoVue Fluor 568 - Phalloidin
- PhenoVue Hoechst 33342 Nuclear stain
- PhenoVue 512 Nucleic acid stain

Note: *Protect stock and staining solutions from light.*

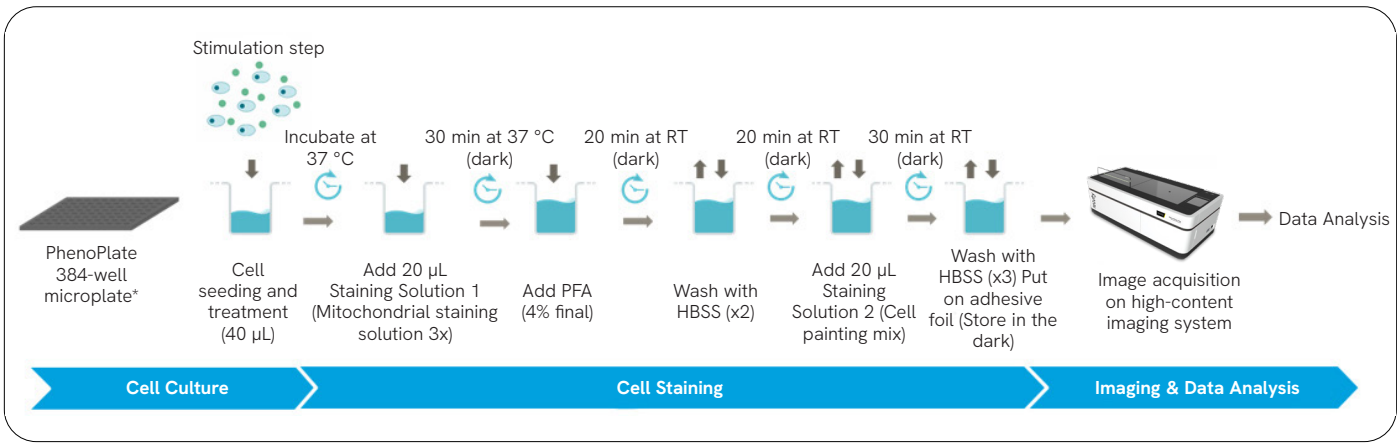
	Reagent name	1. Reconstitution/preparation of stock solution	2. Reconstitution/preparation of staining solutions	Final concentration of reagents per well
	PhenoVue dye diluent A (5x)	Dilute 5 times in distilled H_2O to give a 1x ready to use buffer.	Ready to use for dilution of other reagents.	HBSS + 1 % BSA (1x)
Staining Solution 1	PhenoVue 641 mitochondrial stain	Reconstitute with 40 µL DMSO to give a 1 mM (2000x) stock solution.	Dilute stock solution 666 times in PhenoVue dye diluent A (1x) or cell culture medium to give a 1500 nM (3x) staining solution.	500 nM (272 ng/mL)
Staining Solution 2	PhenoVue Fluor 555 - WGA	Reconstitute with 130 µL 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 600 µL dH_2O to give a 2 mg/mL (400x) stock solution.	Dilute stock solution 400 times in PhenoVue dye diluent A (1x) to give a 5 µg/mL staining solution.	48 nM (5 µg/mL)
	PhenoVue Fluor 568 - Phalloidin	Reconstitute with 60 µL DMSO to give a 6.6 µM (800x) stock solution.	Dilute stock solution 800 times in PhenoVue dye diluent A (1x) to give a 8.25 nM staining solution.	8.25 nM (12 ng/mL)
	PhenoVue Hoechst 33342 nuclear stain	Ready to use stock solution at 1 mg/mL (1000x).	Dilute stock solution 1000 times in PhenoVue dye diluent A (1x) to give a 1 µg/mL staining solution.	1.62 µM (1 µg/mL)
	PhenoVue 512 nucleic acid stain	Ready to use stock solution at 5 mM (833x).	Dilute stock solution 833 times in PhenoVue dye diluent A (1x) to give a 6 µM staining solution.	6 µM (2.86 µg/mL)

Example preparation of staining solutions

The following example describes the preparation of 15 mL Staining Solution 1 and 15 mL Staining Solution 2, sufficient for 1 x 384-well plate.



Experimental workflow



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

Protocol

This protocol references Cimini et al.* (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 48 h.

Note: Depending of the volume of compounds, volume of cells may be adjusted to reach a total 40 µL volume (cells plus compounds).

3. **Add** 20 µL of staining solution 1.
4. **Incubate** for 30 min in the dark at 37 °C, 5% CO_2.

Perform the following steps with no pauses:

5. **Add** 20 µL of 16% (wt/vol) methanol-free PFA (4% final concentration) (vol/vol).
6. **Incubate** at RT for 20 min in the dark.
7. **Wash** two times with 60 µL of 1x HBSS.
8. **Discard** HBSS.
9. **Add** 20 µL of staining solution 2.
10. **Incubate** at RT for 30 min in the dark.
11. **Wash** three times with 60 µL of 1x HBSS.
12. **Do not discard** the final 60 µL HBSS.

Note: HBSS can be supplemented with 0.05% sodium azide if image acquisition is not performed immediately.

13. **Seal the plates** with adhesive foil and store them at 4 °C in the dark until ready to image.
14. **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 Cimini et al.
    - Start the automated imaging sequence according to the microscope manufacturer's instructions.
15. **Image Analysis:** Refer to Cimini et al. for detailed data reduction protocol.

* Cimini BA. et al. Optimizing the Cell Painting assay for image-based profiling. Nat Protoc 18, 1981-2013 (2023).
** 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

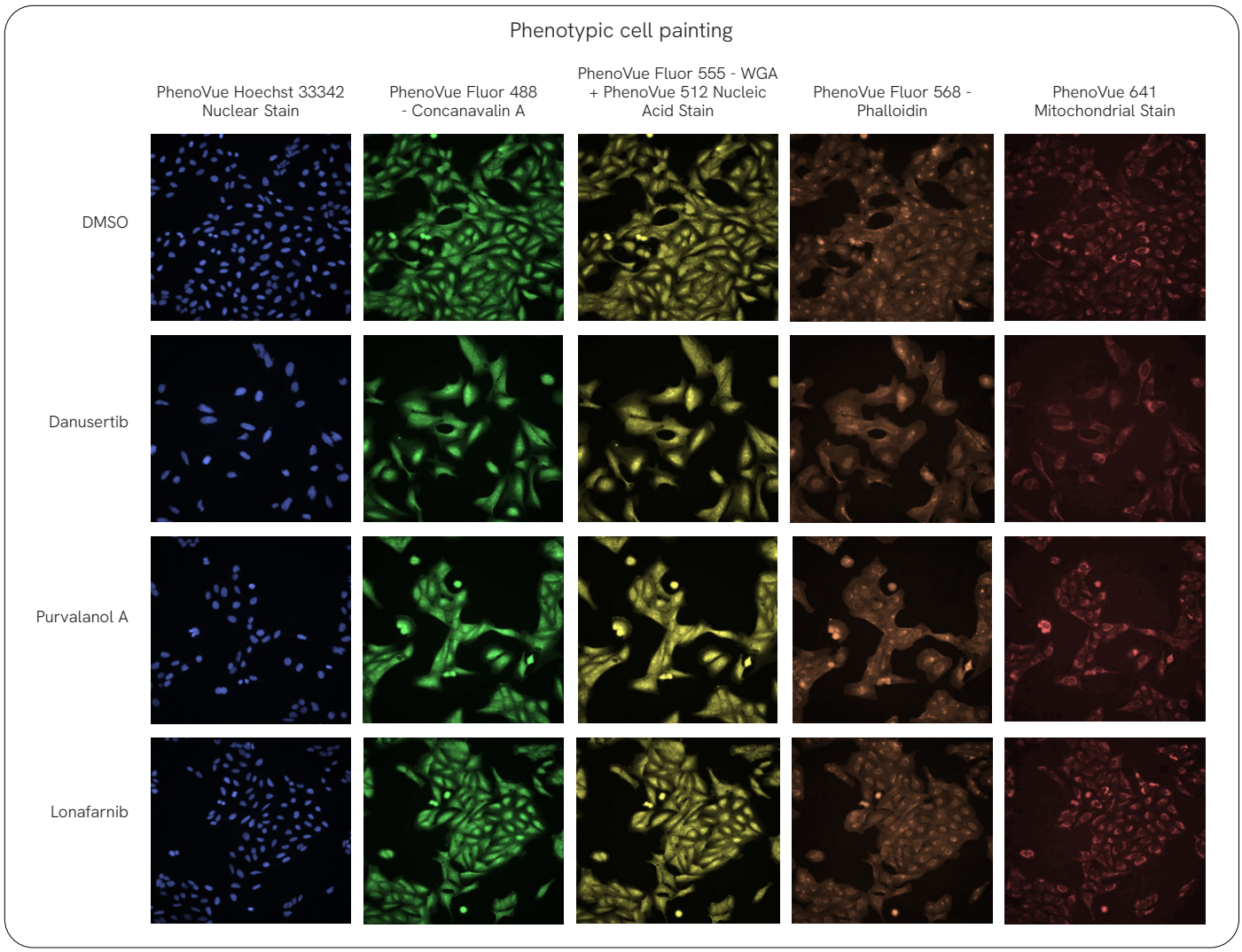


Figure 1: U2OS cells were seeded in PhenoPlate 384-well microplates (1000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were then untreated or treated for 48 h with the indicated compounds prior to applying the cell painting JUMP protocol v3. Images were acquired on the Opera Phenix® high-content screening system.

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