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

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Part Number</th>
<th>Number of Vials per Kit</th>
<th>Shipping Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Cell Painting JUMP Kit - 1 x 384 wells</td>
<td>PING 21</td>
<td>7</td>
<td>Dry ice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Format</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>Lyophilized</td>
<td>1 vial (0.02 mg)</td>
<td>2-8 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue Fluor 488 - Concanavalin A</td>
<td>Lyophilized</td>
<td>1 vial (1.2 mg)</td>
<td>2-8 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - Phalloidin</td>
<td>Dessicated</td>
<td>1 vial (0.4 nmol)</td>
<td>-16 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue 641 Mitochondrial Stain</td>
<td>Dessicated</td>
<td>1 vial (22 µg)</td>
<td>-16 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue Hoechst 33342 Nuclear Stain</td>
<td>Solution in H₂O</td>
<td>1 vial (70 µg, 70 µL)</td>
<td>2-8 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue 512 Nucleic Acid Stain</td>
<td>Solution in DMSO</td>
<td>1 vial (100 nmol, 20 µL)</td>
<td>-16 °C or below. Protect from light.</td>
</tr>
<tr>
<td>PhenoVue Dye Diluent A (5x)</td>
<td>Liquid</td>
<td>1 vial (8 mL)</td>
<td>2-8 °C or below.</td>
</tr>
</tbody>
</table>

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.

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.
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.

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Final Concentration of Reagents Per Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenovue Dye Diluent A (5x)</td>
<td>Dilute 5 times in distilled H₂O to give a 1 mM (2000x) stock solution. Ready to use for dilution of other reagents. HBSS + 1 % BSA (1x)</td>
</tr>
<tr>
<td>Phenovue Fluor 555 - WGA</td>
<td>Reconstitute with 130 µL dH₂O 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. 1.5 µg/ml (43.7 nM)</td>
</tr>
<tr>
<td>Phenovue Fluor 488 - Concanavalin A</td>
<td>Reconstitute (each vial) with 600 µL dH₂O 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. 5 µg/ml (48 nM)</td>
</tr>
<tr>
<td>Phenovue Fluor 568 - Phalloidin</td>
<td>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)</td>
</tr>
<tr>
<td>Phenovue Hoechst 33342 Nuclear Stain</td>
<td>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)</td>
</tr>
<tr>
<td>Phenovue 512 Nucleic Acid Stain</td>
<td>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)</td>
</tr>
</tbody>
</table>
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

Protocol

This protocol has been adapted from Bray et al.* and Cimini et al. (manuscript in preparation).

1. Dispense 40 μL of cells per well into CellCarrier Ultra 384-well microplates** and incubate at 37 °C, 5% CO₂ 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% CO₂ 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% CO₂.

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.


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 60μL 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 Bray et al.
   - Start the automated imaging sequence according to the microscope manufacturer’s instructions.

15. Image Analysis: Refer to Bray et al.*, for detailed data reduction protocol.


**CellCarrier Ultra 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 CellCarrier Ultra 384-well microplates (1000 cells/well) and incubated at 37 °C, 5% CO₂ 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.
Related Products

Opera Phenix Plus High-Content Screening System
Harmony® Imaging and Analysis Software

CellCarrier Ultra Microplates
CellCarrier Ultra ULA-Coated Microplates, 384-well
- Black, clear bottom, ULA-coated, with lid
- Part Number 6057800 / 6057802

CellCarrier Ultra ULA-Coated Microplates, 96-well
- Black, clear bottom, ULA-coated, with lid
- Part Number 6055800 / 6055802

CellCarrier-96 Ultra Microplates
- Black, collagen-coated, with lid
- Part Number 6055700 / 6055708

CellCarrier-96 Ultra Microplates
- Black, PDL-coated, with lid
- Part Number 6055500 / 6055508

CellCarrier-96 Ultra Microplates
- Black, tissue culture-treated, with lid
- Part Number 6055302 / 6055300 / 6055308

CellCarrier-384 Ultra Microplates
- Black with clear bottom, tissue culture-treated, with lid
- Part Number 6057302 / 6057300 / 6057308

CellCarrier-384 Ultra Microplates
- Black, collagen type 1-coated, with lid
- Part Number 6057700 / 6057708

CellCarrier-384 Ultra Microplates
- Black, non-irradiated, tissue culture-treated, with lid
- Part Number 6057328

CellCarrier Microplates
CellCarrier-1536 Microplates
- Black, optically-clear bottom, tissue culture-treated, sterile, with lid
- Part Number 6004550 / 6004558