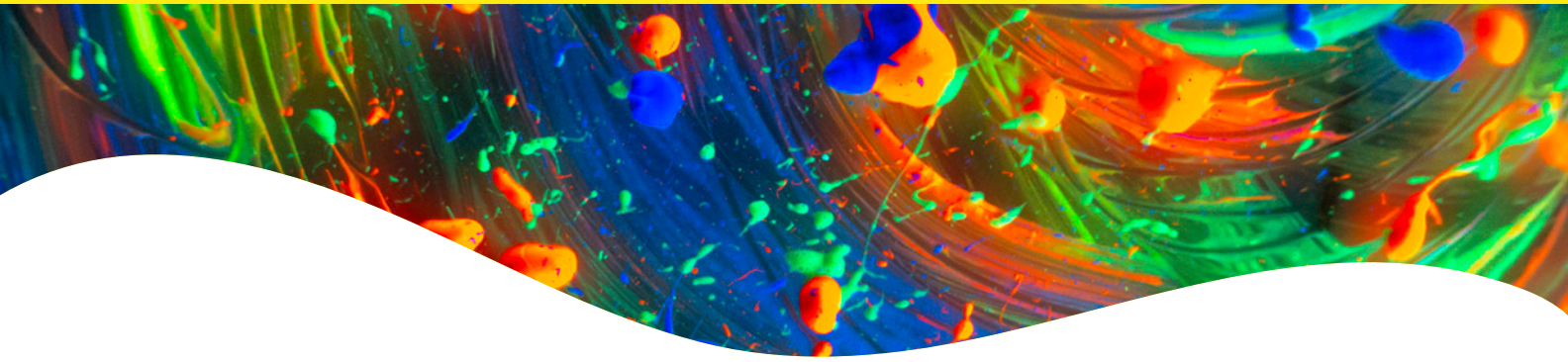
PhenoVue Multi Organelle Staining Kit 1x384



Overview

Mitochondria, lysosomes, lipid droplets, and actin play crucial roles in various cellular processes:

- Mitochondria: often referred to as the “powerhouses” of the cell because their primary function is to generate energy in the form of adenosine triphosphate (ATP) through a process called cellular respiration.
- Lysosomes: membrane-bound organelles responsible for intracellular digestion and waste management.
- Lipid droplets: can be mobilized to release fatty acids when the cell requires energy, or as a means of transporting lipids to different cellular compartments.
- Actin: globular protein that polymerizes to form long, filamentous structures called microfilaments. These microfilaments, collectively known as the actin cytoskeleton, provide structural support to cells and are involved in various cellular processes.

These organelles and cellular components, however, have many more functions and interact with each other in intricate ways to maintain cell homeostasis and carry out specific cellular processes.

Our PhenoVue™ multi organelle staining kit comprises five ready-to-use fluorescent dyes or antibodies to visualize mitochondria, lysosomes, lipid droplets, actin cytoskeleton, and nuclei in a 5-plex experiment. Each component has been extensively validated and carefully optimized, ensuring maximum spectral separation with no spectral overlap, making this kit a straightforward solution to unravel fundamental biological processes, study compounds’ mechanisms of action, and examine disease phenotypes. Applied to paraformaldehyde fixed cellular models, the PhenoVue multi organelle staining kit avoids the use of living cells, increasing your throughput, and providing a convenient method for phenotypic screening approaches such as cell painting. This kit is ideal for the study of metabolic disorders, infectious diseases, and liver toxicity.

Product information

Product name	Part no.	Number of vials per unit	Shipping conditions
PhenoVue multi organelle staining kit	PMOS11	8	Dry ice

Kit contents	Format	Packaging*	Storage
PhenoVue Hoechst 33342 nuclear stain	Liquid (H_2O)	1 vial of 70 µL (50000x)	2-8 °C or below Protect from light
PhenoVue 493 lipid stain	Liquid (DMSO)	1 vial of 30 µL (4000x)	2-8 °C or below Protect from light
PhenoVue Fluor 400LS - phalloidin	Desiccated	1 vial of 30 µL after reconstitution (400X)	-16 °C or below Protect from light
PhenoVue anti-HSP60 antibody 100X	Liquid	1 vial of 100 µL (100x)	-16 °C or below Protect from light
PhenoVue anti-LAMP1 antibody 100X	Liquid	1 vial of 100 µL (100x)	-16 °C or below Protect from light
PhenoVue Fluor 555 - goat anti-mouse antibody highly cross-adsorbed	Liquid	1 vial of 100 µL (100x)	-16 °C or below Protect from light
PhenoVue Fluor 647 - goat anti-rat antibody highly cross-adsorbed	Liquid	1 vial of 50 µL (200x)	-16 °C or below Protect from light
PhenoVue dye diluent A (5X)	Liquid (HBSS + 5%BSA)	1 vial of 8 mL (5x)	-16 °C or below Protect from light

* Amount of reagent provided is sufficient for 1 x 384 wells microplate using the recommended concentrations.

Storage and stability

- 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.
- Allow the reagents to warm up to room temperature for 30 min before opening the vials and reconstitution.
- 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.
- 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.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Common filter set	Quantum yield (ϕ)	Epsilon* (ε in $M^{-1}.cm^{-1}$ at λ max)	Brightness (ϕ x ε)**
PhenoVue Hoechst 33342	357**	455**	DAPI	dsDNA: 0.38 ssDNA: 0.22	43,000	Nd***
PhenoVue 493 lipid stain	493*	504*	FITC	Nd***	88,000	Nd***
PhenoVue Fluor 400LS	395	585	Ex: 375-440 Em: 550-650	Nd***	26,000	Nd***
PhenoVue Fluor 555	555	570	Cy3	0.1	155,000	15,500
PhenoVue Fluor 647	650	670	Cy5	0.3	240,000	72,000

* In methanol ** In methanol with 0.2 M HCl *** Not determined

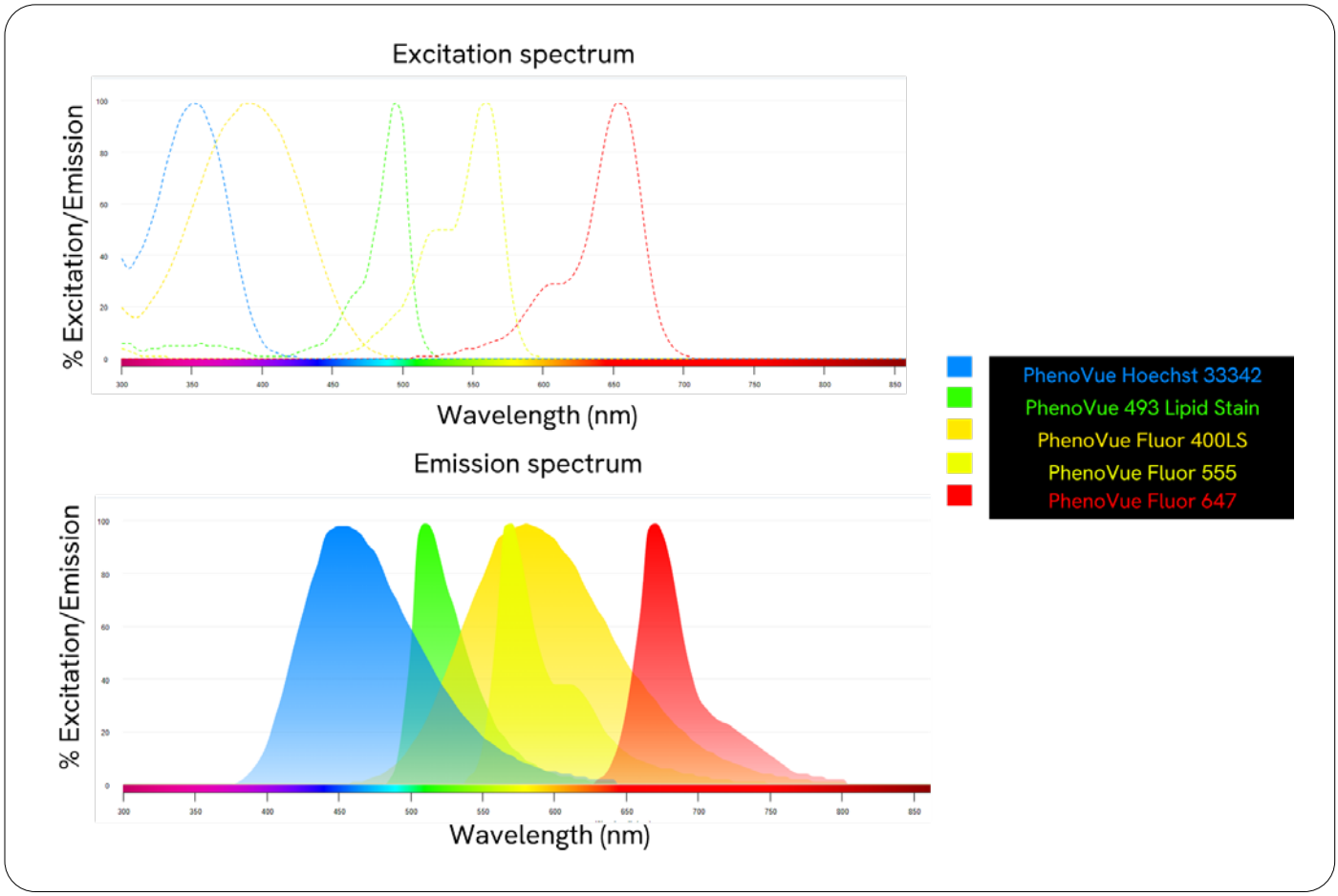


Figure 1: Excitation and emission spectrum associated with the PhenoVue multi organelle kit components.

Other materials and reagents not provided

Reagents or consumables	Usage
PBS	Washing buffer and diluent for bovine serum albumin, protease free and PhenoVue permeabilization 0.5% Triton X-100 solution
PhenoVue paraformaldehyde, 4% solution	Fixation buffer
PhenoVue permeabilization 0.5% Triton X-100 solution	Permeabilization buffer
BSA (bovine serum albumin)	Saturation buffer
DMSO	Reconstitution of PhenoVue Fluor 400LS - phalloidin
PhenoPlate™ 384-well or 96-well	Cell plating, stimulation, staining and imaging
Aluminum single-tab foil	Plate sealing to protect fluorescent probes from light

Reagent reconstitution and preparation of staining solutions

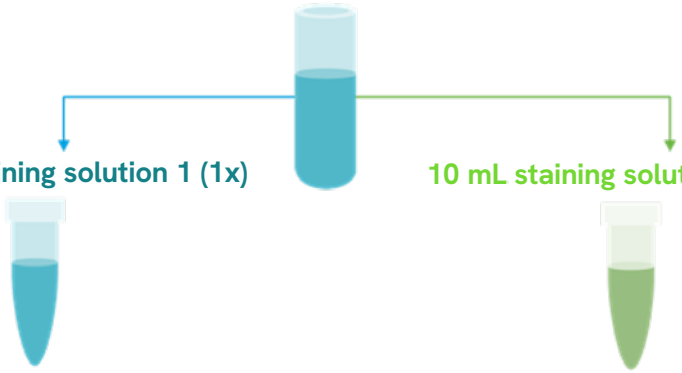
	Reagents	Reconstitution / preparation of stock solution	Preparation of staining solution
Dye diluent preparation	PhenoVue dye diluent A (5X)	Dilute 5 times in distilled H_2O to give a ready to use 1X buffer	Ready to use for dilution of other reagents
Staining solution 1	PhenoVue anti-HSP60 antibody 100X	100X liquid stock solution provided	Dilute stock solution 100 times in PhenoVue dye diluent A to give a 1x staining solution.
	PhenoVue anti-LAMP1 antibody 100X	100X liquid stock solution provided	Dilute stock solution 100 times in PhenoVue dye diluent A to give a 1x staining solution.
Staining solution 2	PhenoVue Hoechst 33342 nuclear stain	50000X liquid stock solution provided (1 mg/mL)	Dilute stock solution 50000 times in PhenoVue dye diluent A to get a 20 ng/mL staining solution. Intermediate dilution is highly recommended (e.g. 250X)
	PhenoVue 493 lipid stain	4000X liquid stock solution provided	Dilute stock solution 4000 times in PhenoVue dye diluent A to give a 1x staining solution
	PhenoVue Fluor 400LS – phalloidin	Desiccated stock solution provided 400X liquid stock solution after reconstitution with 30 µL DMSO	Dilute stock solution 400 times in PhenoVue dye diluent A to give a 1x staining solution
	PhenoVue Fluor 555 - goat anti-mouse antibody highly cross-adsorbed	100X liquid stock solution provided	Dilute stock solution 100 times in PhenoVue dye diluent A to give a 1x staining solution
	PhenoVue Fluor 647 - goat anti-rat antibody highly cross-adsorbed	200X liquid stock solution provided	Dilute stock solution 200 times in PhenoVue dye diluent A to give a 1x staining solution

1. **Prepare stock solution of PhenoVue dye diluent A 1x** as described in the table above.
2. **Prepare permeabilization solution:** Dilute stock solution of PhenoVue permeabilization 0.5% Triton X-100 solution (PVPERM051) 5 times in PBS to give a 0.1% PhenoVue permeabilization Triton X-100 solution (sold separately).
3. **Prepare saturation solution:** Prepare 1% BSA solution diluted in PBS (not provided).
4. **Prepare the staining solutions as described in the table above:** (protect stock and staining solutions from light)
    - **Staining solution 1**: Intended to be used on fixed, permeabilized and saturated cells and includes:
        - PhenoVue anti-LAMP1 antibody
        - PhenoVue anti-HSP60 antibody
    - **Staining solution 2**: Staining solution intended to be used after incubation of the staining solution 1 includes:
        - PhenoVue Hoechst 33342 nuclear stain
        - PhenoVue 493 lipid stain
        - PhenoVue Fluor 400LS – phalloidin
        - PhenoVue Fluor 555 - goat anti-mouse antibody highly cross-adsorbed
        - PhenoVue Fluor 647 - goat anti-rat antibody highly cross-adsorbed

Example preparation of staining solutions

The following example describes the preparation of 10 mL staining solution 1, and 10 mL staining solution 2, sufficient for 1 x 384-well plate (or 2 x 96-well plate).

Prepare 20 mL 1x PhenoVue dye diluent A
To 16 mL ddH_2O, add 4 mL PhenoVue dye diluent A (5x)



10 mL staining solution 1 (1x)

To 9.8 mL PhenoVue dye diluent A (1x)

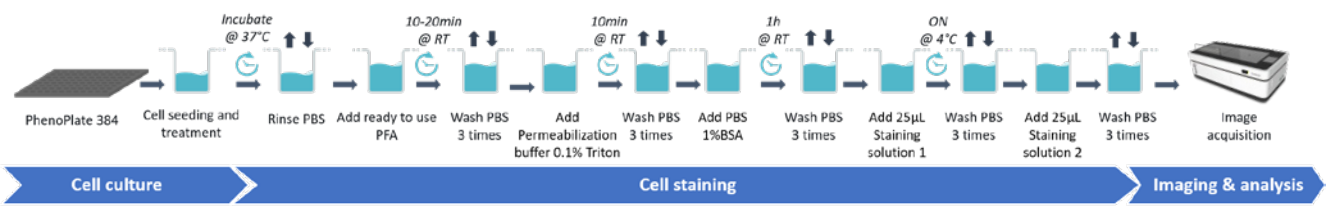
- Add 100 µL of PhenoVue anti-LAMP1 antibody (100x)
- Add 100 µL of PhenoVue anti-HSP60 antibody (100x)

10 mL staining solution 2 (1x)

To 9.8 mL PhenoVue dye diluent A (1x)

- Add 40 µL of PhenoVue Hoechst 33342 nuclear stain (from a 250X intermediate dilution)
- Add 2.5 µL of PhenoVue 493 lipid stain (4000x)
- Add 25 µL of PhenoVue fluor 400LS – phalloidin (400x)
- Add 100 µL of PhenoVue Fluor 555 - Goat Anti-Mouse Antibody Highly Cross-Adsorbed (100x)
- Add 50 µL of PhenoVue Fluor 647 - Goat Anti-Rat Antibody Highly Cross-Adsorbed (200x)

Experimental workflow



Protocol

Cell culture

Seed cells in PhenoPlate 384-well microplates* 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.

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 10-20 min at room temperature (not provided).
2. **Washing:** Wash three times with PBS.
3. **Permeabilization:** Add PhenoVue Permeabilization solution 0.1% Triton X-100 for 10 min at room temperature (not provided).
4. **Washing:** Wash three times with PBS for 5 min.
5. **Saturation:** Incubate with PBS-1% BSA for 1h at room temperature (not provided).
6. **Washing:** Wash three times with PBS for 5 min.
7. **Primary antibody incubation:** Add 25 µL per well of Staining Solution 1 and incubate for overnight at 4°C.
8. **Washing:** Wash three with PBS for 5 min.
9. **Staining solution:** Add 25 µL per well of Staining Solution 2 and incubate for 1h at room temperature protected from light.
10. **Washing:** Wash three times with PBS for 5 min.
11. Acquire images on an imaging device.

** PhenoPlate 96-well microplates may also be used. Adjust the cell density accordingly and increase the corresponding volumes by 2-fold.*

Tips

- Fluorescent dye concentrations have been carefully optimized to limit fluorescence crosstalk on Revvity's high content screening instruments. Thus, increasing reagent concentrations may increase crosstalk.
- Using PhenoPlate 384-well microplates, centrifuge the plate for 1 min (500 g) at room temperature between each step to help reagents settle in the bottom of the wells.
- For the reconstitution step of PhenoVue Fluor 400LS – phalloidin, 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.
- Staining can be performed on human and mouse cellular models, except the LAMP1 antibody which is human specific.
- Perform sequential image acquisition of Hoechst 33342 (Ex: 405/425 nm, Em: 435-480 nm) and PhenoVue Fluor 555/568 (Ex: 561 nm; Em: 570-630 nm) channels to avoid crosstalk from PhenoVue Fluor 400LS - Phalloidin in the PhenoVue Fluor 555 channel.

Recommendations for acquisition settings

- The PhenoVue multi organelle staining kit enables multiplexing of up to 5 colors simultaneously. If using a Revvity high content screening system, we recommend the following acquisition settings (depending on the instrument), to obtain high fluorescent signal as well as best image quality:

HCS instruments		PhenoVue Hoechst 33342	PhenoVue Fluor 400LS - phalloidin	PhenoVue 493 lipid stain	PhenoVue Fluor 555	PhenoVue Fluor 647
Opera Phenix® Plus 5 lasers	Excitation laser	375	425	488	561	640
	Emission filters	435-480	570-630	500-550	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation laser	405	405	488	561	640
	Emission filters	435-480	570-630	500-550	570-630	650-760
Operetta® CLS™ 8 LED - 1600	Excitation LED (filters)	370 (355-385)	405 (390-420)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filters	430-500	570-650	500-550	570-650	655-760
Operetta CLS 8 LED - 1601	Excitation LED (filters)	370 (355-385)	440 (435-460)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filters	430-500	600-640 or 570-650	500-550	570-650	655-760
Operetta CLS 4 LED	Excitation LED (filters)	370 (355-385)	370 (355-385)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filters	430-500	570-650	500-550	570-650	655-760

For simultaneous acquisition (e.g. Opera Phenix™ Plus high-content imaging system):

Perform sequential image acquisition of Hoechst 33342 (Ex: 405/425 nm, Em: 435-480 nm) and PhenoVue Fluor 555/568 (Ex: 561 nm; Em: 570-630 nm) channels to avoid crosstalk from PhenoVue Fluor 400LS - Phalloidin in the PhenoVue Fluor 555 channel.

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

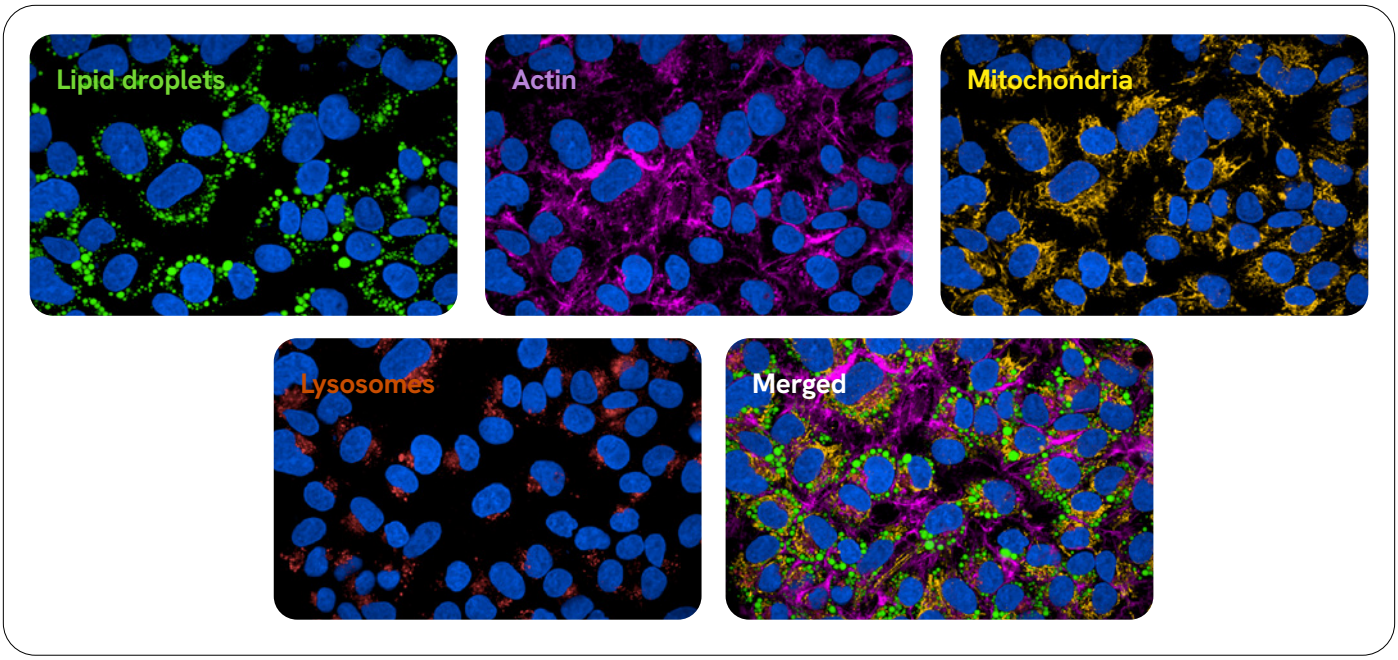


Figure 2: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were treated with oleic acid 200 µM for 24h to induce lipid droplets formation. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT), permeabilized (PhenoVue Permeabilization 0.1% Triton X-100 Solution – 10 min at RT) and stained using the PhenoVue multi organelle staining kit (protocol described above). Images were acquired on the Opera Phenix Plus (5 lasers) high-content screening system with the 63X water objective.

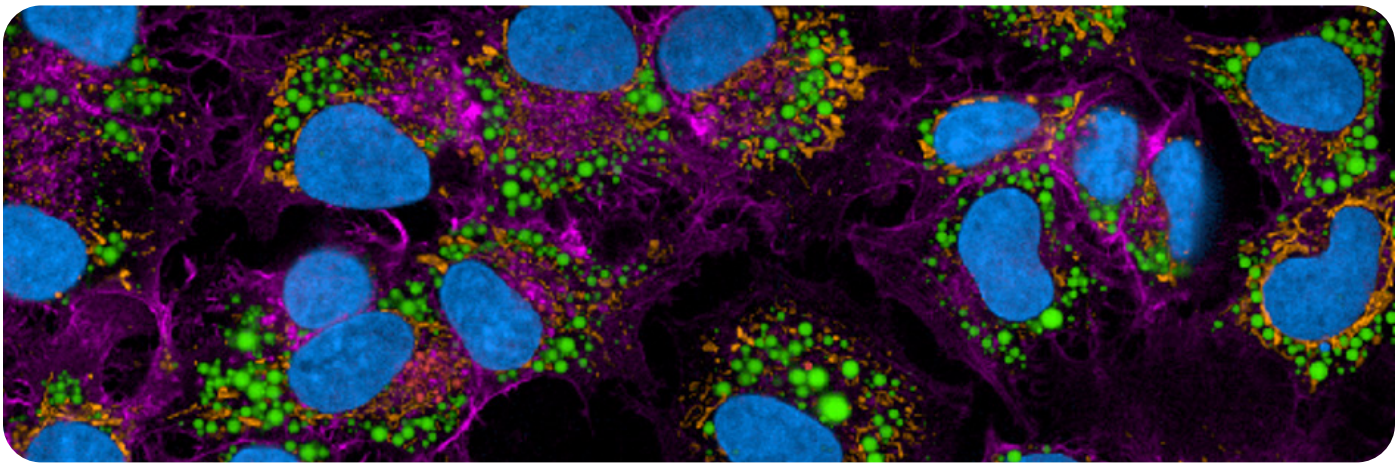


Figure 3: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were treated with oleic acid 200 µM for 24h to induce lipid droplets formation. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT), permeabilized (PhenoVue Permeabilization 0.1% Triton X-100 Solution – 10 min at RT) and stained using the PhenoVue multi organelle staining kit (protocol described above). Images were acquired on the Operetta CLS 8LED 1800 high-content analysis system with the 63X water objective.

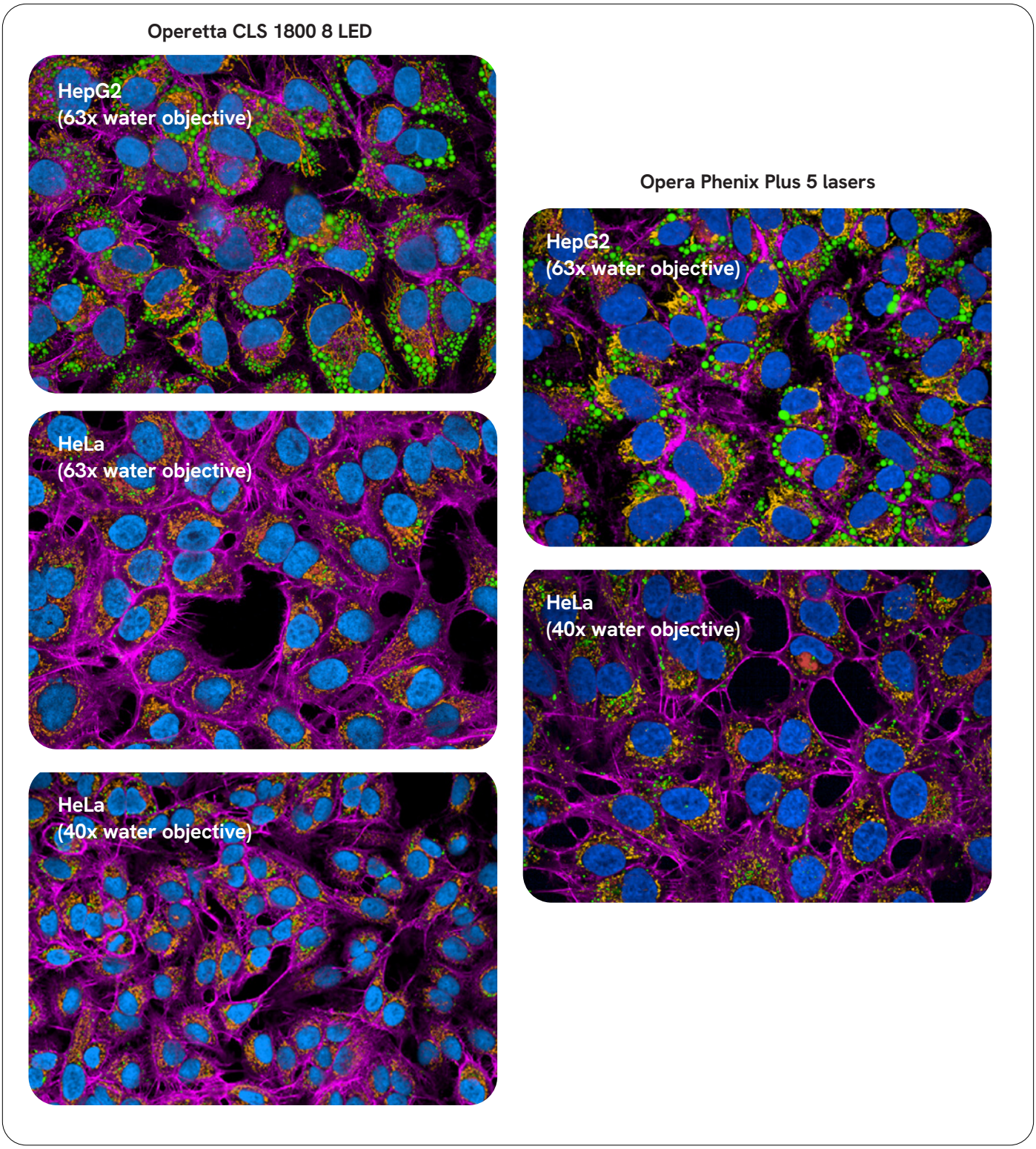


Figure 4: HepG2 or HeLa cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT), permeabilized (PhenoVue Permeabilization 0.1% Triton X-100 Solution – 10 min at RT) and stained using the PhenoVue multi organelle staining kit (protocol described above). Images were acquired either on the Operetta CLS 8LED 1800 high-content analysis system.

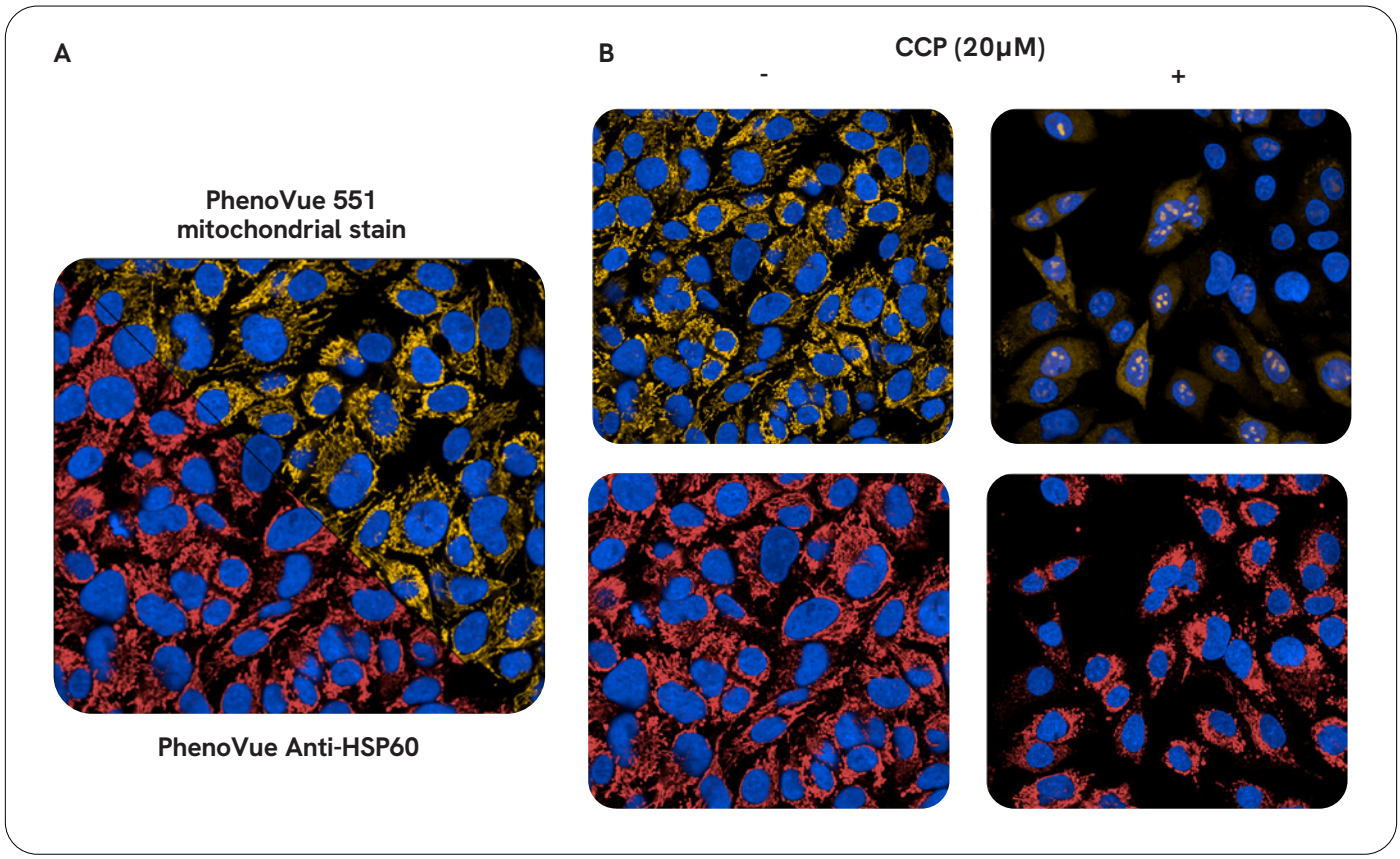


Figure 5: A-Typical tubular mitochondrial shapes with both PhenoVue anti- HSP60 antibody (Red) and PhenoVue 551 mitochondrial stain (Orange) B-To assess mitochondria specific staining of anti- HSP60 antibody, HepG2 cells were treated or not with CCCP compound which disrupts mitochondria membrane potential. As expected, CCCP treatment induces a decrease of the orange, fluorescent signal staining obtained with the PhenoVue 551 mitochondrial stain (sensitive to mitochondrial membrane depolarization) while the red fluorescent staining associated with anti-HSP60 antibody / PhenoVue Fluor 647 Goat anti-Mouse antibody remains unchanged.

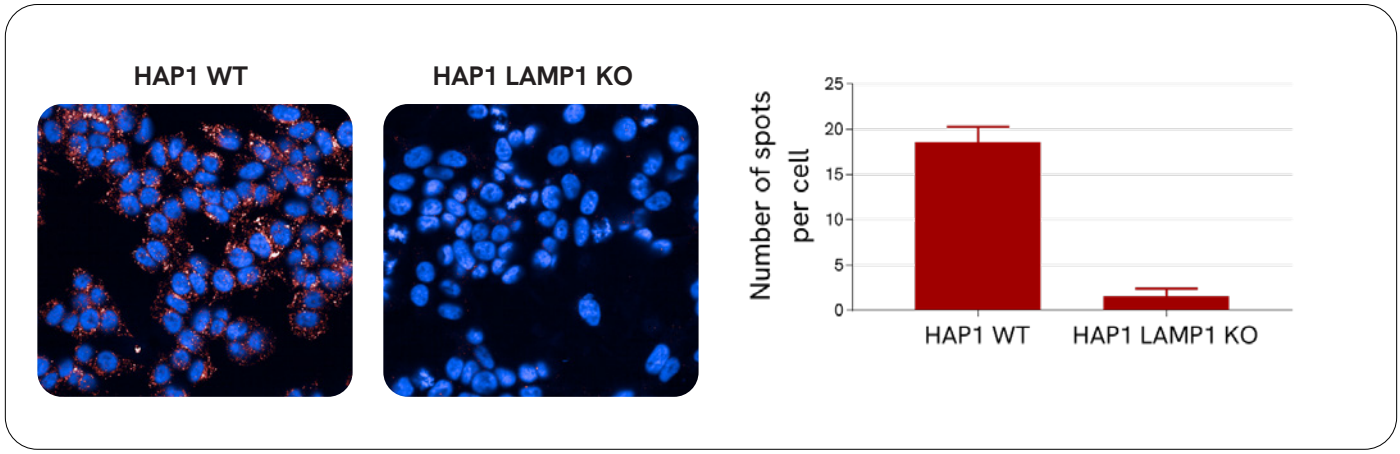


Figure 6: HAP1 WT and HAP1 LAMP1 KO were stained with PhenoVue anti- LAMP1 antibody and PhenoVue Fluor 647 Goat anti-Rat Highly Cross Adsorbed antibody. As expected, no fluorescent signal was detected in the LAMP1 Knock-Out cells, validating the specificity of PhenoVue anti- LAMP1 antibody.

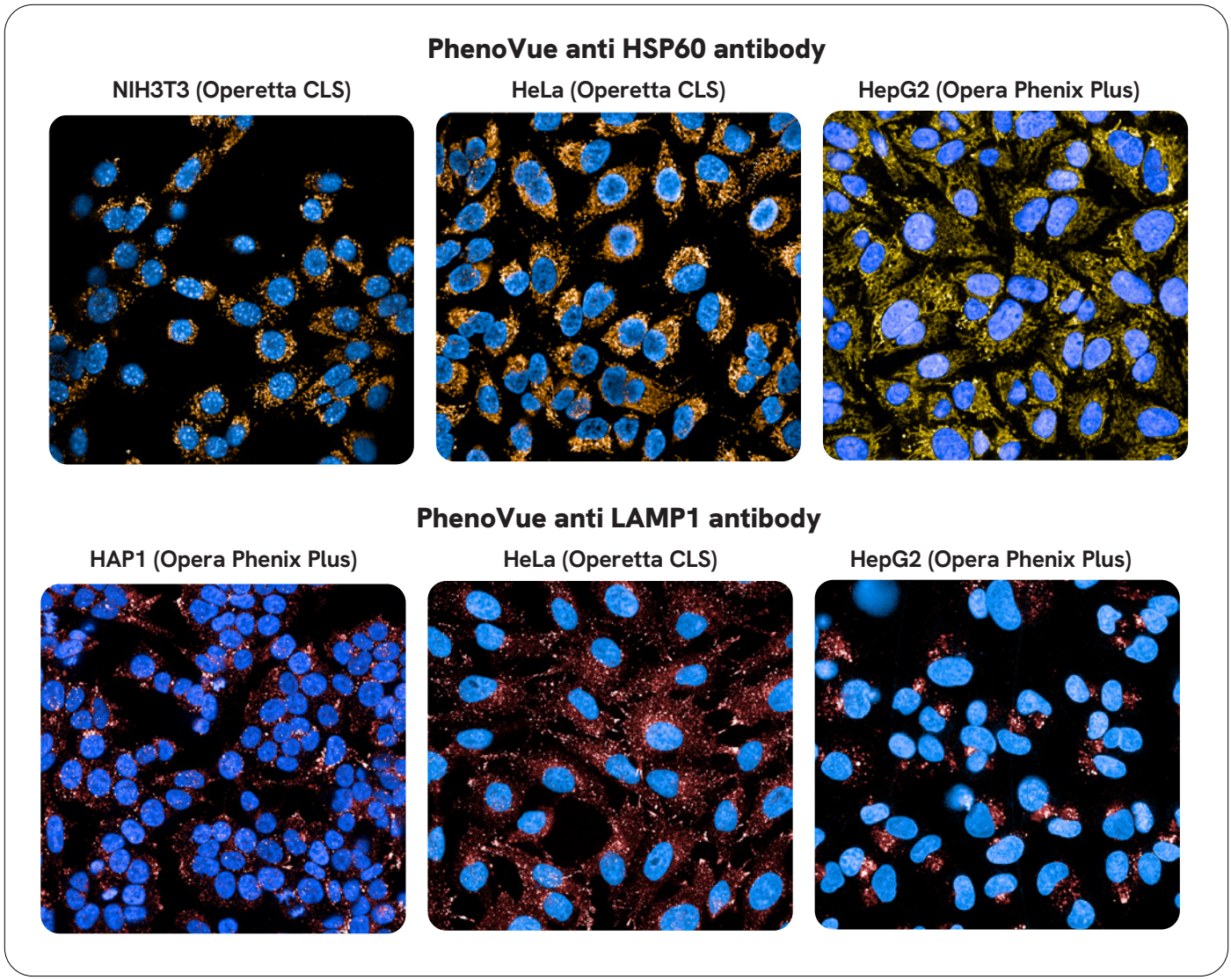


Figure 7: Various cellular models were co-stained with PhenoVue anti-HSP60 associated with PhenoVue Fluor 555 Goat anti-Mouse antibody Highly Cross-Adsorbed, PhenoVue anti-LAMP1 associated with PhenoVue Fluor 647 Goat anti-Rat antibody Highly Cross-Adsorbed, as well as PhenoVue Hoechst 33342. Images were acquired either on the Operetta CLS or Opera Phenix Plus HCS instruments (63X objective).

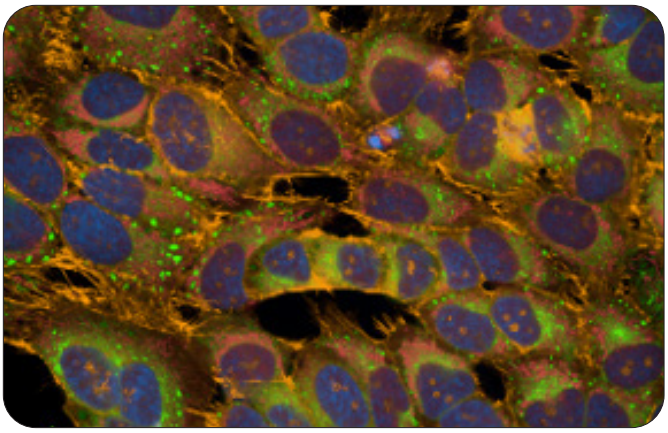


Figure 8: Phenotypic cell painting. HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated with a 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 for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system.

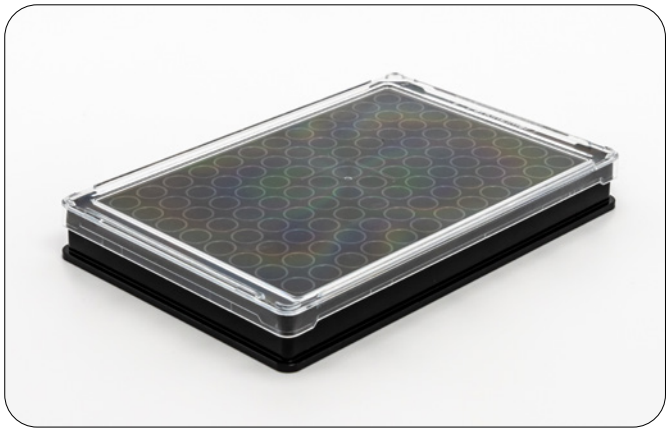


Figure 9: PhenoPlate 96-well microplate

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