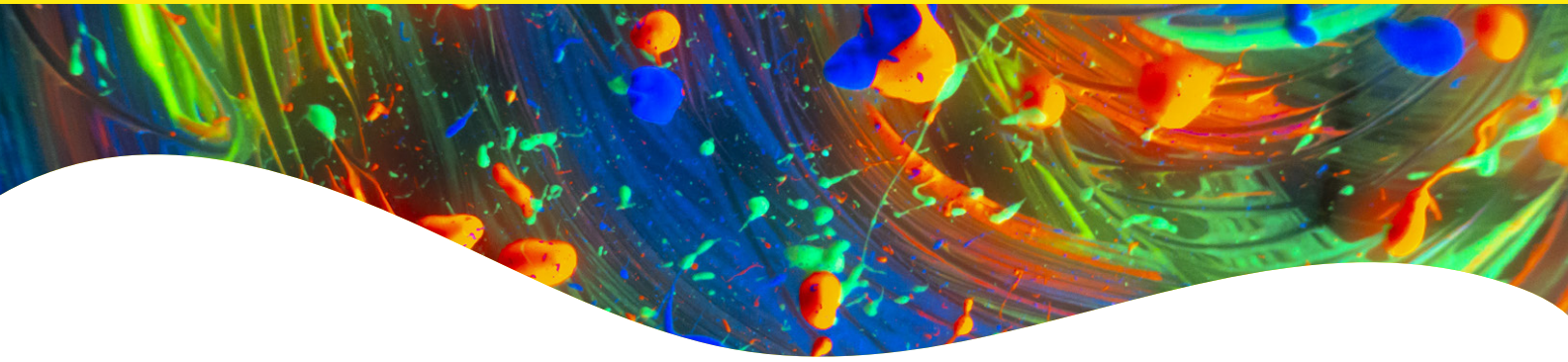
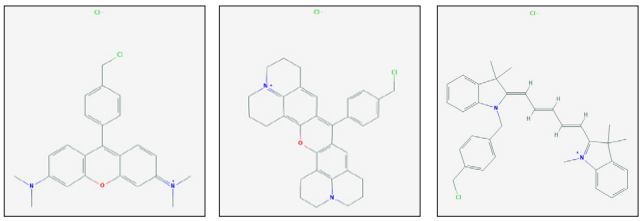
PhenoVue Mitochondrial Stains



Overview

Mitochondria are essential double membrane-bound cell organelles, often referred to as 'the powerhouse of the cell', which provide cellular energy in the form of ATP. Mitochondria play a role in many processes such as cellular metabolism, calcium homeostasis, cell death as well as cell differentiation and growth. Mitochondrial functions are maintained through the mitochondrial membrane potential which reflects the process of electron transport and oxidative phosphorylation, leading to energy production. Mitochondrial dysfunction, which encompasses impaired mitochondrial biogenesis, dynamics and trafficking, Ca^{2+} imbalance, oxidative stress as well as mitophagy, is also associated with several human diseases, such as neurodegenerative disorders.



Structure of PhenoVue 551 Mitochondrial Stain. Source: PubChem CID 25195394

Structure of PhenoVue 578 Mitochondrial Stain. Source: PubChem CID 22613925

Structure of PhenoVue 641 Mitochondrial Stain. Source: PubChem CID 25195433

PhenoVue™ mitochondrial stains are cationic rosamine or cyanine based structures. With mitochondrial membrane potential, they accumulate in mitochondria through electrostatic interactions and react with thiol moieties forming stable thioether bonds. Therefore PhenoVue Mitochondrial stains are well retained after cell fixation.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue 551 mitochondrial stain	CP3O1	20	50 µg (117 nmoles)	Dessicated	Dry ice
PhenoVue 578 mitochondrial stain	CP3R1	20	50 µg (94 nmoles)	Dessicated	Dry ice
PhenoVue 641 mitochondrial stain	CP3D1	20	50 µg (92 nmoles)	Dessicated	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 15 mins before opening the vials and reconstitution.
- After reconstitution, aliquoted reagents must be stored at -16 °C or below and are stable for 3 months.

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working concentration range*
PhenoVue 551 mitochondrial stain	427.4 g/mol	Reconstitution using 117 µL anhydrous DMSO gives a stock concentration of 1 mM (427 µg/mL)	25 – 500 nM (10.7 ng/mL – 213.7 ng/mL)
PhenoVue 578 mitochondrial stain	531.5 g/mol	Reconstitution using 94 µL anhydrous DMSO gives a stock concentration of 1 mM (531 µg/mL)	25 – 500 nM (13.3 ng/mL – 265 ng/mL)
PhenoVue 641 mitochondrial stain	543.6 g/mol	Reconstitution using 92 µL anhydrous DMSO gives a stock concentration of 1 mM (543 µg/mL)	25 – 500 nM (13.6 ng/mL – 271 ng/mL)

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

Equivalent number of microplates

Product name	When used at recommended concentration	96-well Microplate (100 µL - 300 µL per Well)	384-well Microplate (25 µL - 90 µL per Well)	1536-well Microplate (4 µL - 12 µL per Well)
PhenoVue 551 mitochondrial stain	100 nM (42.7 ng/mL)	Approx. 800 to 2400	Approx. 650 to 2400	Approx. 1250 to 3800
PhenoVue 578 mitochondrial stain	100 nM (53 ng/mL)	Approx. 650 to 1950	Approx. 550 to 1950	Approx. 1000 to 3050
PhenoVue 641 mitochondrial stain	100 nM (53 ng/mL)	Approx. 650 to 1950	Approx. 550 to 1950	Approx. 1000 to 3050

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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 551 mitochondrial stain	551	576	Cy3 / RFP	nd**	102000	nd**
PhenoVue 578 mitochondrial stain	578	599	Texas Red	nd**	117000	nd**
PhenoVue 641 mitochondrial stain	641	662	Cy5	nd**	194000	nd**

* In methanol ** Not determined

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue 551 mitochondrial stain	Yes	Yes	No
PhenoVue 578 mitochondrial stain	Yes	Yes	No
PhenoVue 641 mitochondrial stain	Yes	Yes	No

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.

Fixed-cell imaging

1. First, follow the live-cell imaging protocol as described below, then proceed with cell fixation if required for subsequent manipulation, e.g., application of additional stains.
2. **Fixation:** 2 options:
   1. 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.
      or
   2. Add 100% methanol (chilled to -20 °C) at room temperature for 5 min.
3. **Washing:** Wash three times with PBS.
4. **Permeabilization** (if required for subsequent manipulation, e.g., application of additional stains):
   a. For PFA fixed cells, add ready to use PhenoVue permeabilization 0.5% Triton X-100 solution (PVPERM051) for 10 min (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.
   b. Methanol fixed cells do not require permeabilization.
5. **Washing:** Wash three times with PBS for 5 min.
6. **Optional:** Incubate with 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.
7. **Washing:** Wash once with PBS for 5 min.
8. Acquire images on an imaging device.

Live-cell imaging

1. Rinse briefly in HBSS.
2. Incubate with 25 nM-500 nM PhenoVue mitochondrial stain for 15-45 min at RT.
3. Rinse in HBSS.
4. Acquire images on a live cell imaging device.

Note that cytotoxicity of staining reagents such as Hoechst 33342 is usually observed in long term imaging.

Tips

- Permeabilization with ice-cold acetone (5 min) can improve the specific fluorescence signal by reducing the background.
- To assess mitochondrial membrane potential, it is preferable to use PhenoVue 551 mitochondrial stain or PhenoVue 578 mitochondrial stain which are more sensitive to variations of mitochondrial membrane potential.
- PhenoVue 551 mitochondrial stain, PhenoVue 578 mitochondrial stain and PhenoVue 641 mitochondrial stain can be used for mitochondria localization and quantification, as well as multiplexing experiments.
- PhenoVue 551, 578 and 641 mitochondrial stains are comparable to Mitotracker™ Orange CMTMRos, Mitotracker™ Red CMXRos and Mitotracker™ Deep-Red stains.

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

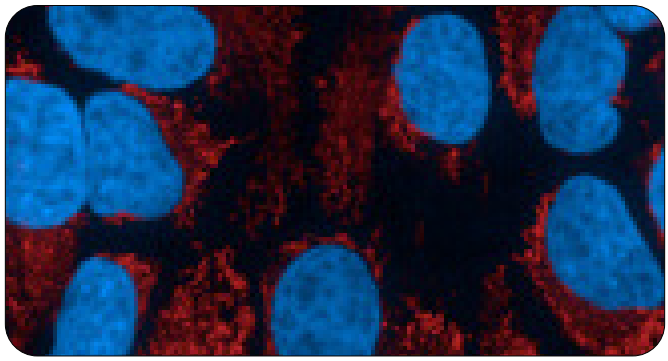

Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Live cells were stained with 150 nM of **PhenoVue 641 mitochondrial stain** for 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS™ high-content analysis system.

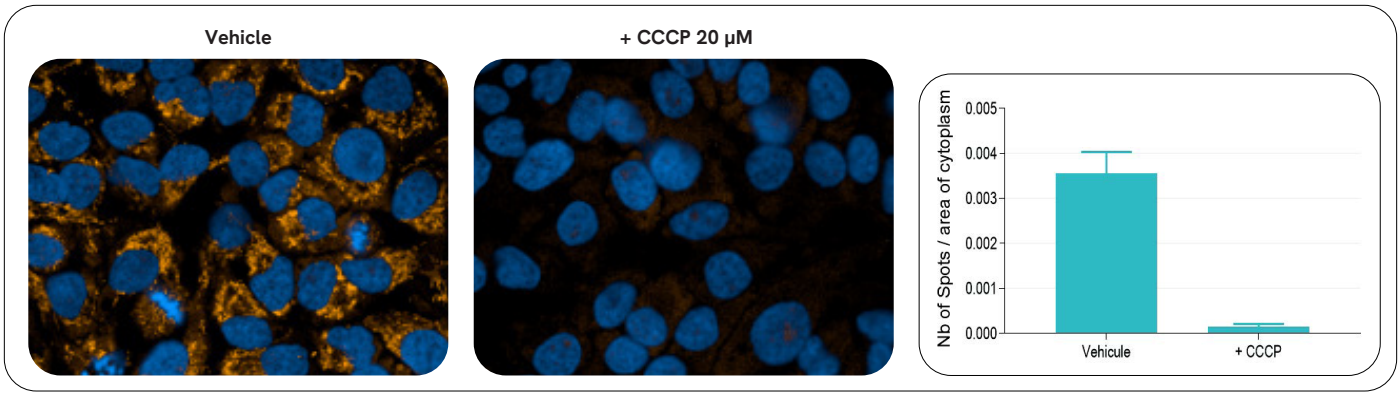

Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were untreated or treated with CCCP (20 µM, 60 min), shown to disrupt the mitochondrial membrane potential. Live cells were stained with 100 nM **PhenoVue 551 mitochondrial stain** for 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS high-content analysis system.

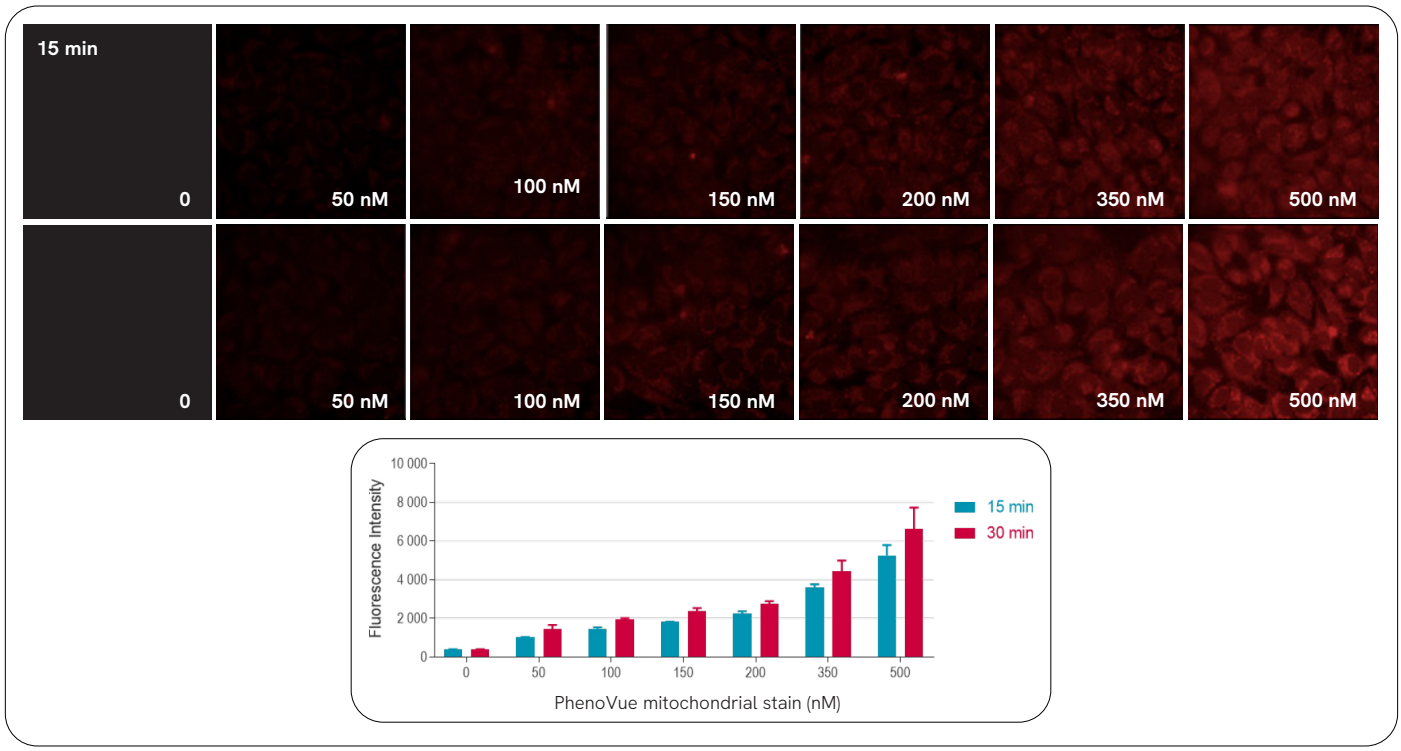

Figure 3: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Live cells were stained with increasing concentrations of **PhenoVue 578 mitochondrial stain** for 15 or 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS high-content analysis system.

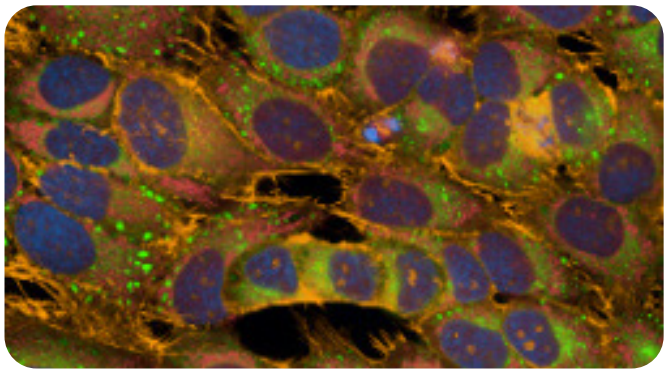

Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 Mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated with a Cell Painting mix 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.

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