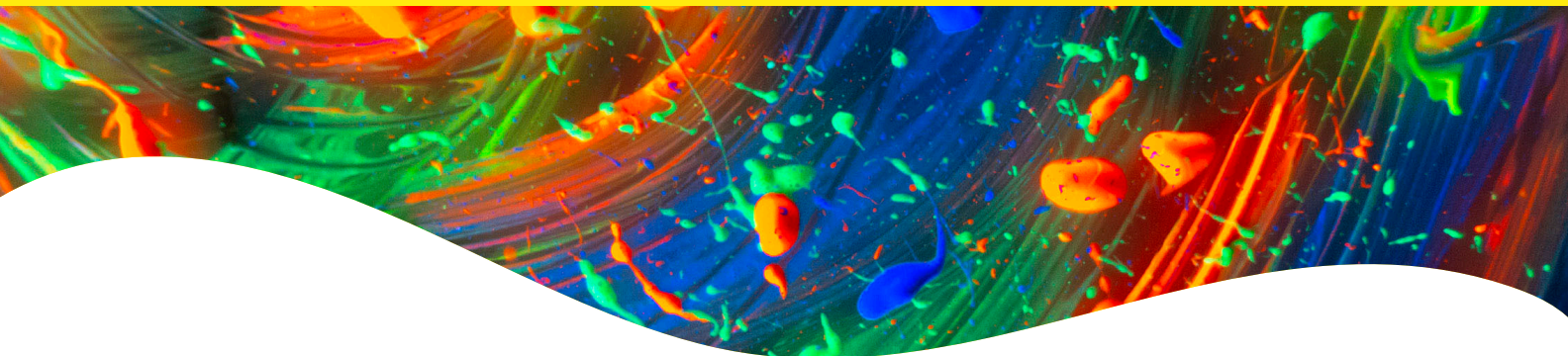


# PhenoVue Lysosomal Stains



## Overview

Lysosomes are abundant organelles which contain more than sixty acidic enzymes. Lysosomes play essential role in macromolecules and organelles break down through the autophagy-lysosomal system. Defective regulation of lysosomal function has been reported in several diseases such as lysosomal storage disorders and neurodegenerative diseases like Alzheimer's and Parkinson's.

PhenoVue™ lysosomal stains are partially protonated at neutral pH, and highly protonated at acidic pH. In living cells, PhenoVue lysosomal stains accumulate in acidic vesicles such as lysosomes, resulting in bright and specific lysosomal fluorescent staining.

## Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue 503 lysosomal stain	CP10G1	20	50 µL (18.1 µg)	Solution 1mM DMSO	Dry ice
PhenoVue 577 lysosomal stain	CP10R1	20	50 µL (19.9 µg)	Solution 1mM DMSO	Dry ice
PhenoVue 633 lysosomal stain	CP10DR1	10	5.5 µg	Desiccated	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 aliquote.
- Aliquoted reagents must be stored at -16 °C or below and are stable for 3 months.

## Equivalent number of microplates

Product name	When used at recommended concentration	96-well microplate (100 $\mu$ L - 300 $\mu$ L per well)	384-well microplate (25 $\mu$ L - 90 $\mu$ L per well)	1536-well microplate (4 $\mu$ L - 12 $\mu$ L per well)
PhenoVue 503 lysosomal stain	18.1 ng/mL	Approx. 650 to 2050	Approx. 550 to 2050	Approx. 1050 to 3250
PhenoVue 577 lysosomal stain	19.96 ng/mL	Approx. 650 to 2050	Approx. 550 to 2050	Approx. 1050 to 3250
PhenoVue 633 lysosomal stain	0.055 $\mu$ g/mL	Approx. 3 to 10	Approx. 3 to 10	Approx. 5 to 16

View our full range of high-quality imaging microplates at [Revvity.com](https://www.revvity.com)

## Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working concentration range*
PhenoVue 503 lysosomal stain	398.7 g/mol (as HCl salt)	Provided in solution at 1 mM DMSO	9 ng/mL - 27 ng/mL
PhenoVue 577 lysosomal stain	435.7 g/mol (as HCl salt)	Provided in solution at 1 mM DMSO	10 ng/mL - 30 ng/mL
PhenoVue 633 lysosomal stain	-**	Reconstitution using 100 $\mu$ L dH <sub>2</sub> O gives a stock concentration of 1000X	55 $\mu$ g/mL

\* Dilutions can be done in PBS, HBSS, PhenoVue dye diluent A or cell culture medium. \*\* Information not provided

## Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)*	Maximum emission wavelength (nm)*	Common filter set	Epsilon* ( $\epsilon$ in M <sup>-1</sup> .cm <sup>-1</sup> at $\lambda$ . max)
PhenoVue 503 lysosomal stain	503	510	FITC	74000
PhenoVue 577 lysosomal stain	577	590	RFP	86000
PhenoVue 633 lysosomal stain	633	655	Cy5	-**

\* In methanol. \*\* Information not provided.

## Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue 503 lysosomal stain	Yes	No	No
PhenoVue 577 lysosomal stain	Yes	No	No
PhenoVue 633 lysosomal stain	Yes	No	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<sub>2</sub> until 50-70% confluency.

### Live-cell imaging

1. (Optional) Rinse briefly in HBSS.
2. Incubate with 25-75 nM PhenoVue 503 or 577 lysosomal stain, or 1X PhenoVue 633 lysosomal stain for 30 min to 2 h at RT or at 37 °C, 5% CO<sub>2</sub>.
3. (Optional) Hoechst 33342 staining can be performed with PhenoVue lysosomal stains.
4. (Optional) Rinse in HBSS.
5. 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 experiments.

## Tips

- PhenoVue lysosomal stains can be used for lysosomes localization and quantification, as well as multiplexing experiments.
- Decrease of lysosomal fluorescence intensity after staining with PhenoVue lysosomal stains and LysoTracker stains has been observed after multiple washing steps and over time.

## 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

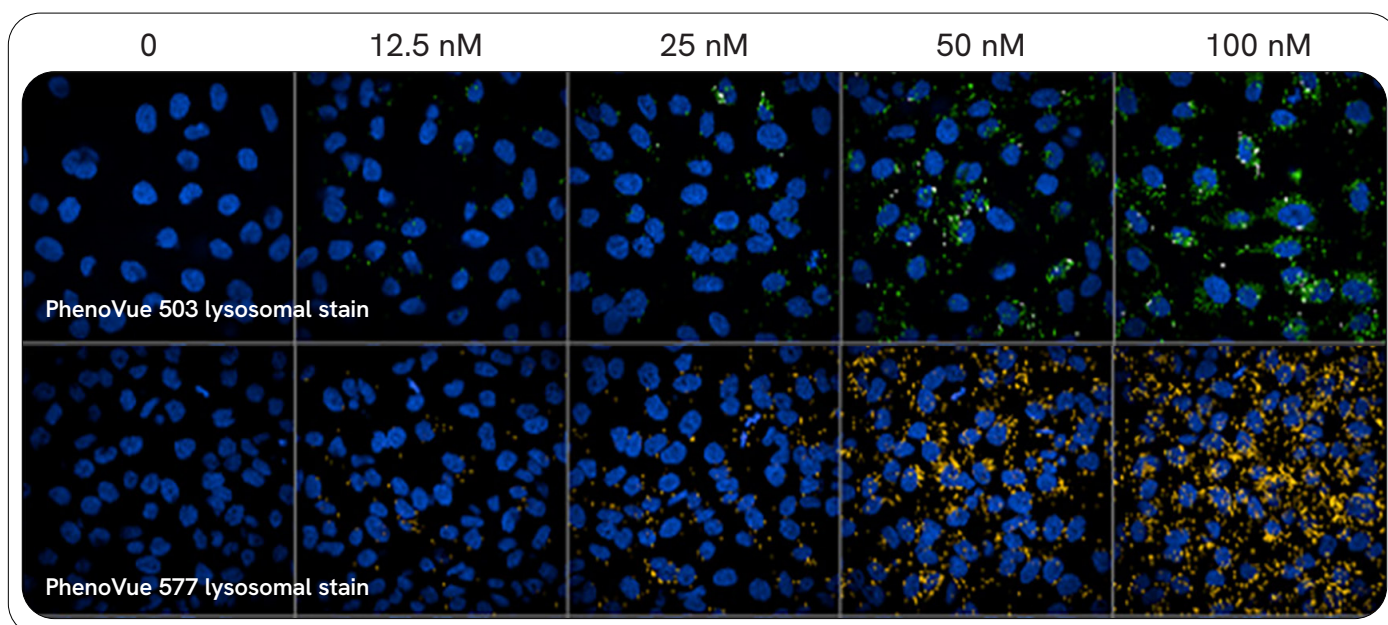


Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Live cells were stained PhenoVue Hoechst 33342 (5µg/mL) for 10 min then incubated with increasing concentrations of **PhenoVue 503 or 577 lysosomal stains** and at 37 °C for 30 min. Images were acquired on the Operetta® CLS™ high-content analysis system.

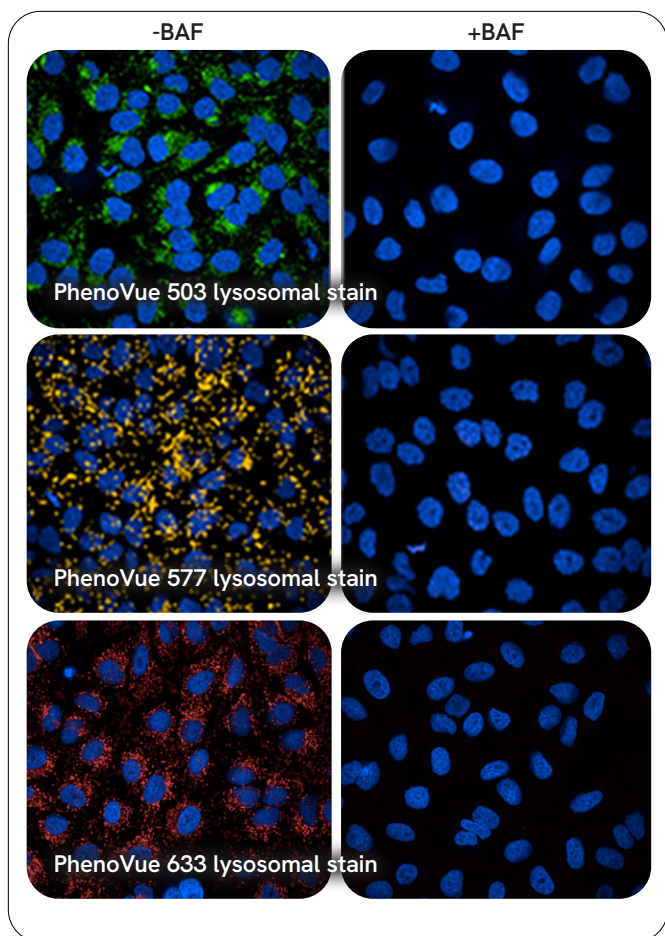


Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were treated with 0.5 μM Bafilomycin A for 1 hour, then stained PhenoVue Hoechst 33342 (5 μg/mL) for 10 min prior to incubation with 25 nM **PhenoVue 503, 577 lysosomal stains (25 nM)** or **PhenoVue 633 lysosomal stain (1X)** and at 37 °C for 30 min. Images were acquired on the Operetta CLS high-content analysis system.