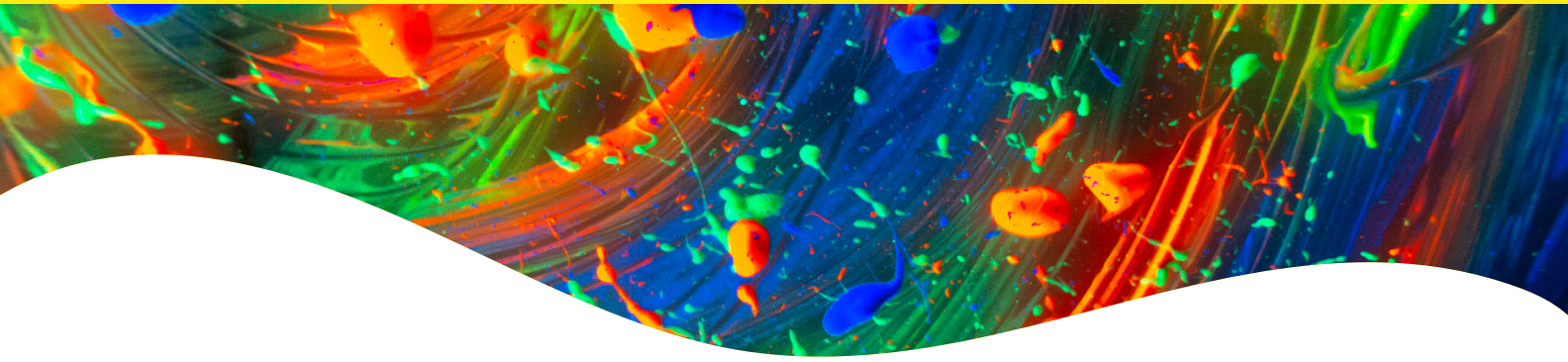
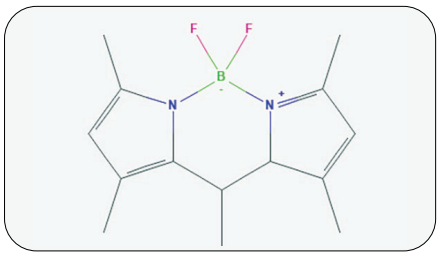
PhenoVue Nile Red Lipid Stain



Overview

Nile red is a lipophilic organic molecule that is almost nonfluorescent in water and polar solvents. In lipid-rich environments, Nile red exhibits enhanced yellow fluorescence, as well as red fluorescence to a lesser extent. Nile red is commonly used for localization and quantification of intracellular lipid droplets.



Structure of PhenoVue™ Nile red lipid stain. Source: PubChem CID 65182.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Nile red lipid stain	CP41	2	10 mg (31.4 µmoles)	Powder	RT

Storage and stability

- Store powder at room temperature or 2 - 8 °C, protected from light.
- 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 powder to warm up to room temperature for 15 min before opening the vials and reconstitution.
- After reconstitution, aliquoted reagents must be stored at - 16 °C or below and are stable for 6 months. Avoid repeated freeze / thaw cycles.

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working concentration range*
PhenoVue Nile red lipid stain	318.4 g/mol	Reconstitution using 31.4 mL anhydrous DMSO gives a stock concentration of 1 mM (318.4 ng/mL)	100 nM – 1000 nM (0.03 ng/mL – 0.32 ng/mL)

* Dilutions can be done in PBS.

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 Nile red lipid stain	200 nM (0.008 ng/mL)	Approx. 10900 to 32700	Approx. 9090 to 32700	Approx. 17040 to 40900

View our full range of high-quality imaging microplates at Revvity.com

Spectral and photophysical properties

PhenoVue Nile red lipid stain complexed with	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)
Triglycerides	512	585	Cy3.5	nd**	43000	nd**
Phospholipids	552	638	Texas Red	nd**	43000	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 Nile red lipid stain	Yes	Yes	Yes

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. For lipid droplet staining, it is preferable to use charcoal stripped FBS to minimize the level of lipid-related components that are usually present in 10% serum supplemented media.

PhenoVue Nile red lipid stain is compatible with live as well as fixed and permeabilized cells. However, the fixation step may change the morphology of lipid droplets.

Fixed-cell imaging

1. 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.

2. Washing: Wash three times with PBS.

3. Permeabilization:

1. 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.
2. Methanol fixed cells do not require permeabilization.

4. Washing: Wash three times with PBS for 5 min.

5. Staining: Incubate with 100-1000 nM PhenoVue Nile red lipid stain for 15-30 min at RT.

6. Washing: Wash three times with PBS for 5 min.

7. Optional: Incubate with 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.

8. Washing: Wash once with PBS for 5 min.

9. Acquire images on an imaging device.

Live-cell imaging

1. Rinse briefly in HBSS.

2. Incubate with 100-1000 nM PhenoVue Nile red lipid stain for 15-30 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

- PhenoVue Nile red lipid stain is compatible with live & fixed cells. However, fixation step may change the morphology of lipid droplets.
- While a bright fluorescence of PhenoVue Nile red lipid stain is emitted at 585 nm with lipid droplets containing triglycerides, PhenoVue Nile red lipid stain may also react with phospholipids inserted in membranes leading to Red-shifted fluorescence emission (638 nm). Therefore, multiplexing experiments with PhenoVue Nile red lipid stain are not recommended.
- For multiplexing experiments, it is preferable to use PhenoVue 493 lipid stain.
- Structures of PhenoVue Nile red lipid stain and PhenoVue 493 lipid stain are shown to the right.

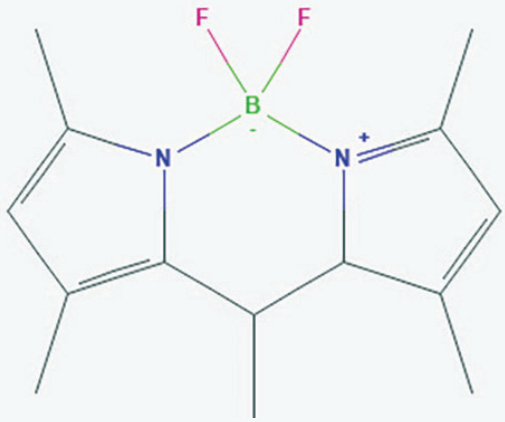
Safety information

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

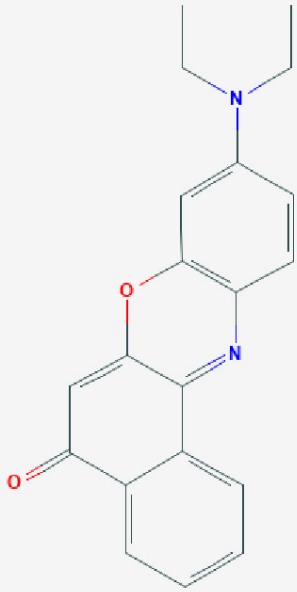
- High-content analysis / high-content screening
- Imaging microscopy
- Flow cytometry

PhenoVue 493 lipid stain



Source: PubChem CID 134716599

PhenoVue Nile red lipid stain



Source: PubChem CID 65182

Validation data

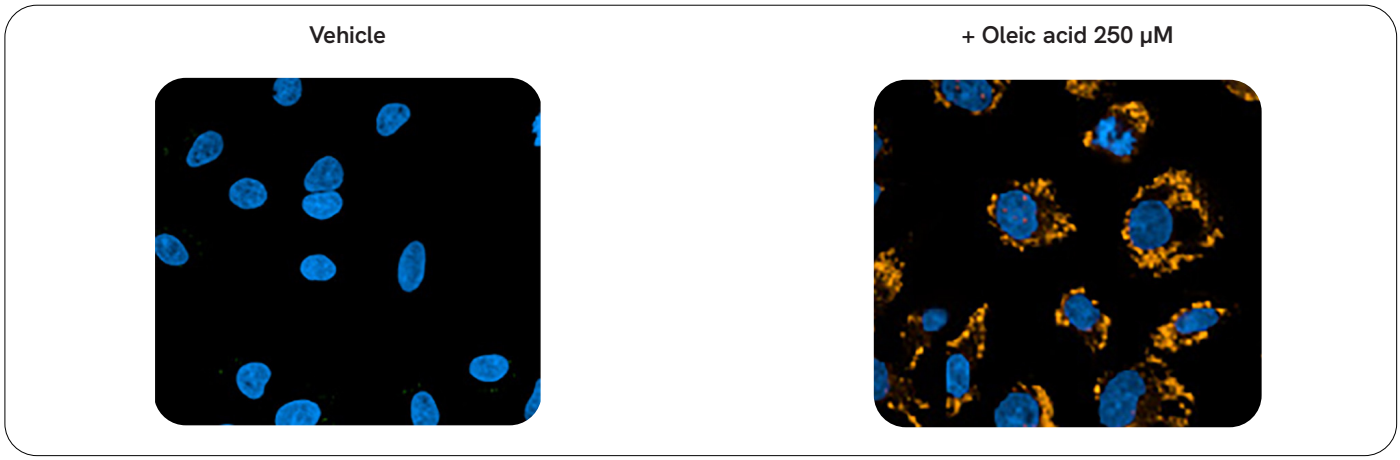
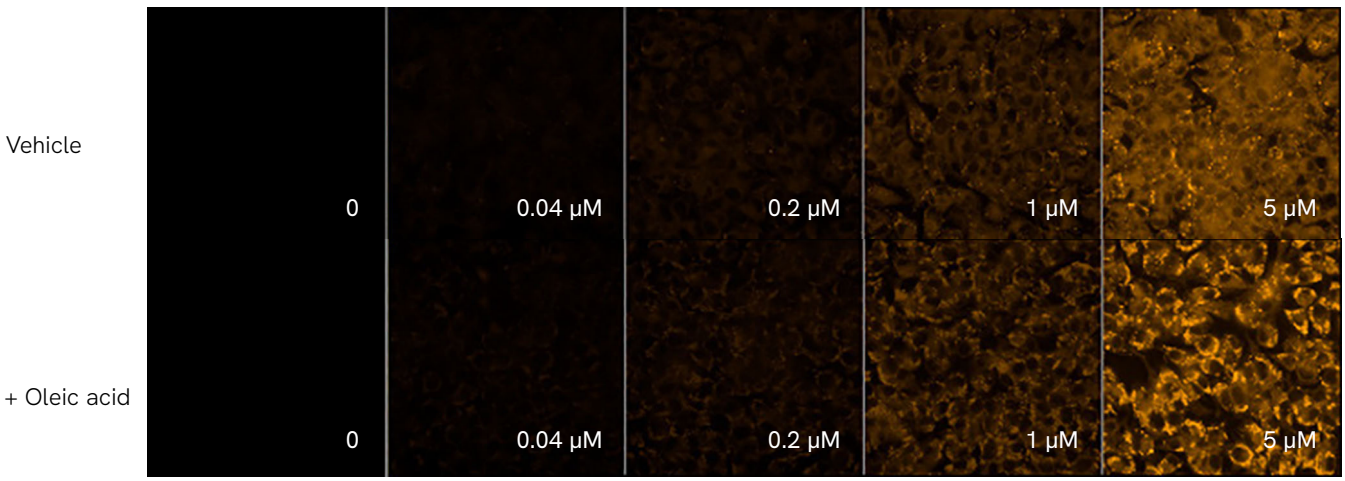


Figure 1: HepG2 cells were seeded in PhenoPlate™ 96-well microplates (20,000 cells/well) and incubated in cell culture medium supplemented with 10% charcoal stripped BS at 37 °C, 5% CO_2 for 24h. Cells were untreated or treated with Oleic acid-BSA complex (250 µM, 24h), shown to stimulate lipid droplet formation. Cells were fixed then permeabilized and stained with 200 nM of **PhenoVue Nile red lipid stain** for 30 min at RT. Nuclei were stained with 5 µg/mL **PhenoVue Hoechst 33342 nuclear stain**. Images were acquired on the Operetta CLS™ high-content analysis system. Note that charcoal stripped FBS is used to minimize the level of lipid-related components such as hormones that are usually present in 10% serum supplemented media.



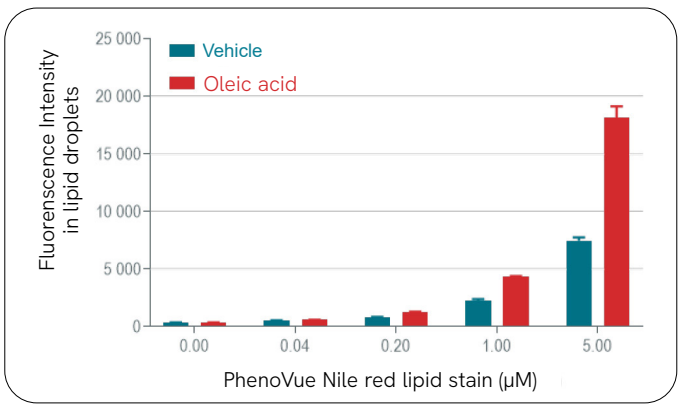


Figure 2: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated in cell culture medium supplemented with 10% charcoal stripped FBS at 37 °C, 5% CO_2 for 24h. Cells were untreated or treated with Oleic acid-BSA complex (250 µM, 24h), shown to stimulate lipid droplet formation. Cells were fixed then permeabilized and stained with increasing concentrations of **PhenoVue Nile red lipid stain** for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system. Note that charcoal stripped FBS is used to minimize the level of lipid-related components such as hormones that are usually present in 10% serum supplemented media.

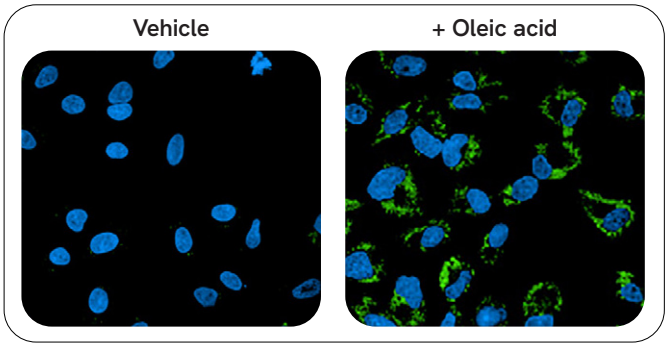


Figure 3: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated in cell culture medium supplemented with 10% charcoal stripped FBS at 37 °C, 5% CO_2 for 24h. Cells were untreated or treated with Oleic acid (250 µM, 24h), shown to stimulate lipid droplet formation. Cells were fixed then permeabilized and stained with 1 µM of **PhenoVue 493 lipid stain** for 30 min at RT. Nuclei were stained with 5 µg/mL **PhenoVue Hoechst 33342 nuclear stain**. Images were acquired on the Operetta CLS high-content analysis system. Note that charcoal stripped FBS is used to minimize the level of lipid-related components such as hormones that are usually present in 10% serum supplemented media.

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