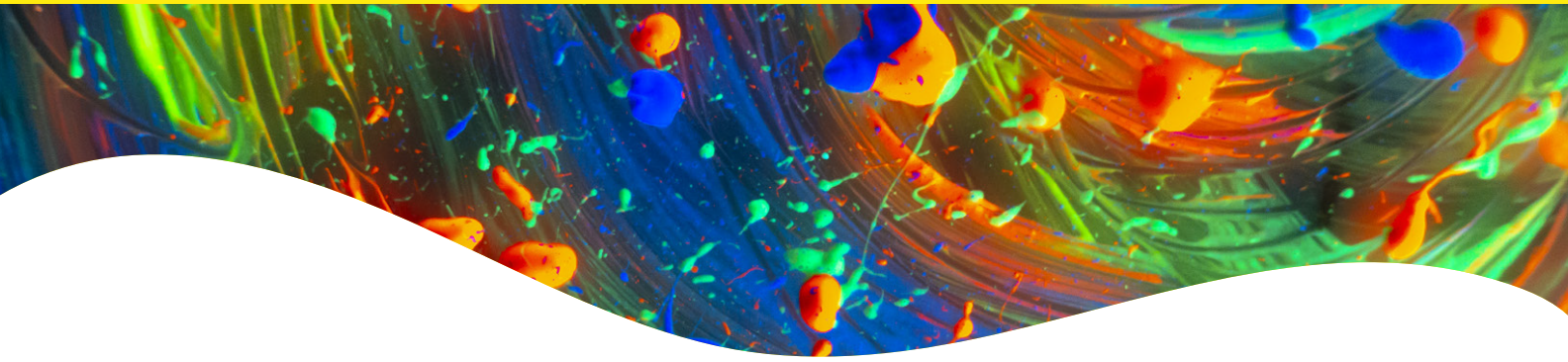
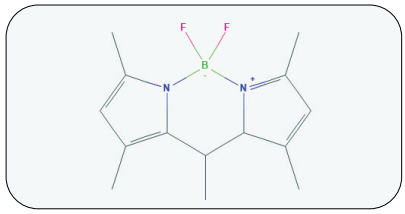
# PhenoVue 493 Lipid Stain



## Overview

PhenoVue™ 493 lipid stain is a lipophilic fluorescent organic molecule that localizes to polar lipids.

PhenoVue 493 lipid stain can be used for localization and quantification of intracellular lipid droplets.



Structure of PhenoVue 493 lipid stain.
Source: PubChem CID 134716599

### Product information

| Product name | Part no. | Number of vials per unit | Quantity per vial | Format | Shipping conditions |
|---|---|---|---|---|---|
| PhenoVue 493 lipid stain | CP51 | 5 | 2 mg<br>4 mM (1.05 g/L) | Solution in 1.95 mL DMSO | Dry ice |

### Storage and stability

- Store stock solution 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 reagent to thaw at room temperature before opening the vials.
- Aliquoted reagents must be stored at -16 °C or below and are stable for 6 months.

## Recommended reconstitution

| Product name | Molecular weight | Recommended stock concentration | Working concentration range* |
|---|---|---|---|
| PhenoVue 493 lipid stain | 262.11 g/mol | Already reconstituted in 1.95 mL anhydrous DMSO to give a stock concentration of 4 mM (1.05 g/L) | 1 µM - 10 µM (0.26 mg/L - 2.6 mg/L) |

* 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 493 lipid stain | 5 µM (1.31 mg/L) | Approx. 270 to 830 | Approx. 230 to 830 | Approx. 430 to 1300 |

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

## 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 493 lipid stain | 493 | 504 | FITC | nd** | 88000 | 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 493 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 493 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 1-10 µM PhenoVue 493 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 1-10 µM PhenoVue 493 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 493 lipid stain is compatible with live & fixed cells. However, the fixation step may change the morphology of lipid droplets.
- PhenoVue 493 lipid stain is comparable to BODIPY™ 493/503.
- Structures of PhenoVue 493 lipid stain and PhenoVue Nile red lipid stain are shown to the right

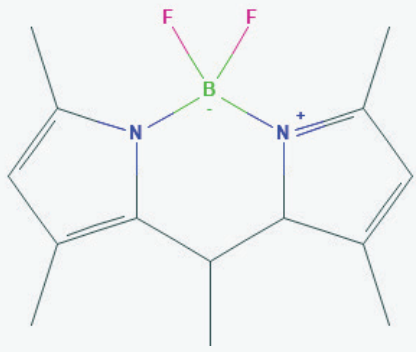
## Safety information

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

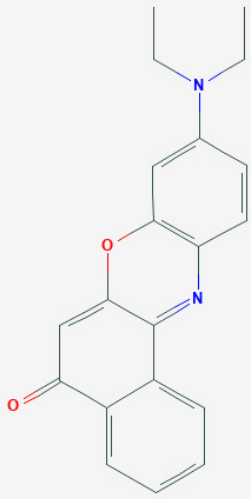
- High-content analysis/high-content screening
- Imaging microscopy
- Flow cytometry

**PhenoVue 493 Lipid stain**




Source: PubChem CID 134716599

**PhenoVue Nile Red Lipid stain**




Source: PubChem CID 65182

## Validation data

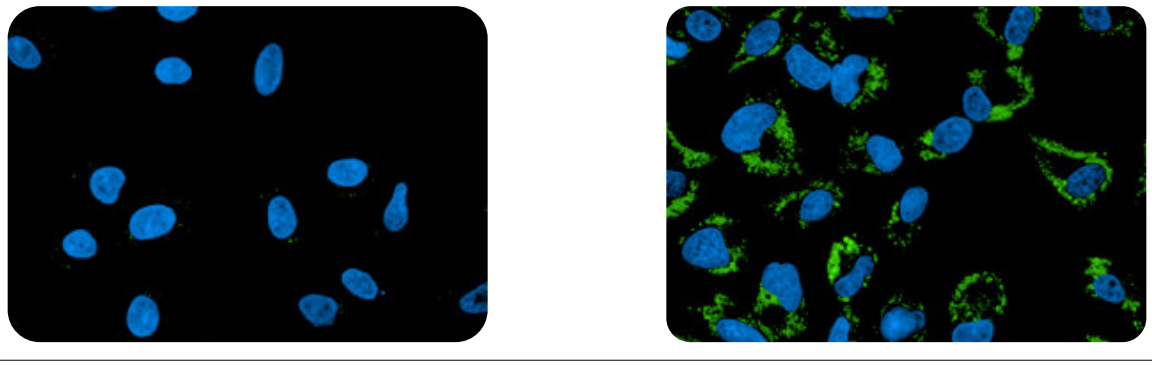



Figure 1: HepG2 cells were seeded in PhenoPlate™ 96-well microplates (20,000 cells/well) and incubated in cell culture medium supplemented with 10% charcoal stripped FBS at 37 °C, 5% $CO_2$ for 24h. Cells were untreated or treated with oleic acid (250 µM, 24h), shown to stimulate lipid droplet formation. 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.

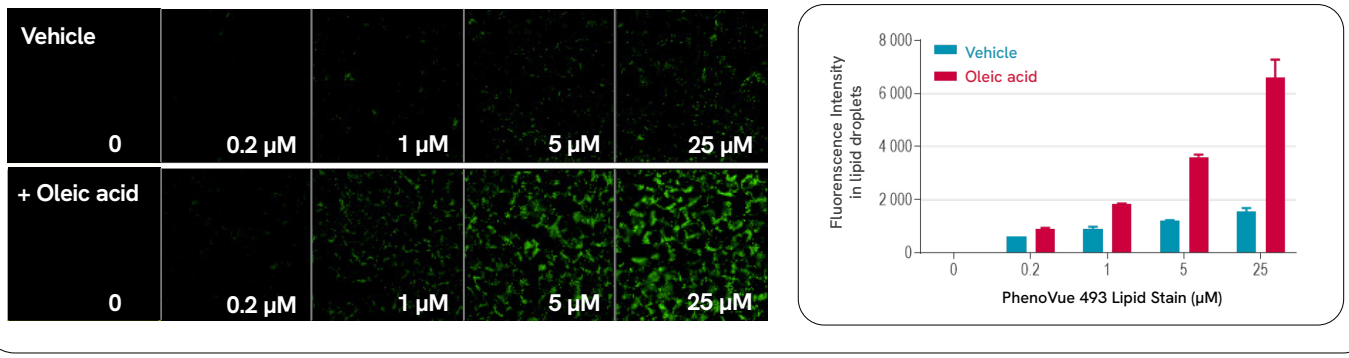



Figure 2: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated in cell culture medium supplemented with 10% charcoal stripped FBS at 37 °C, 5% $CO_2$ for 24h. Cells were untreated or treated with oleic acid (250 µM, 24h), shown to stimulate lipid droplet formation. Cells were fixed then permeabilized and stained with increasing concentrations of **PhenoVue 493 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.

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