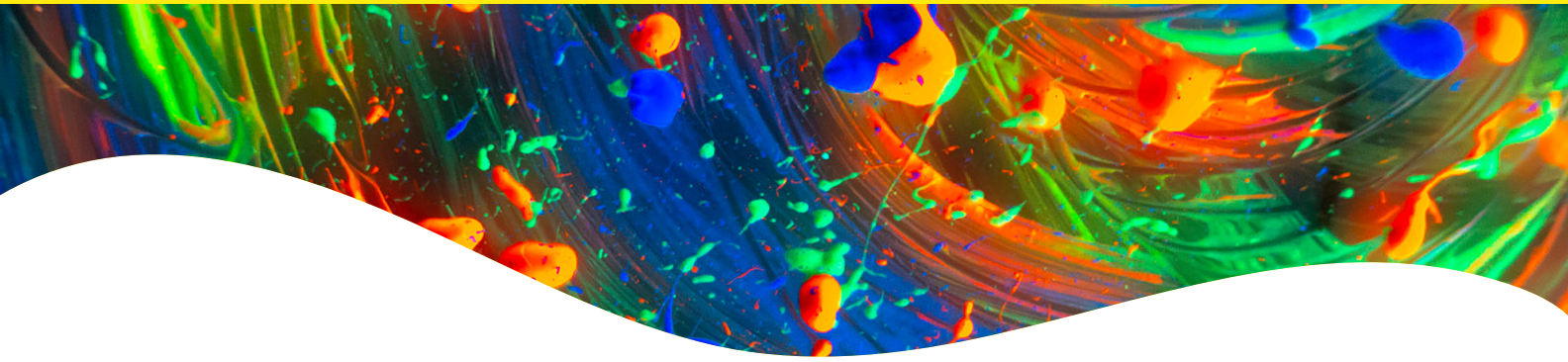
PhenoVue 512 Nucleic Acid Stain



Overview

PhenoVue™ 512 nucleic acid stain is a cell permeable organic molecule which binds nucleic acids. It displays a higher fluorescence intensity when complexed with RNA compared to DNA.

PhenoVue 512 nucleic acid stain concentrates into RNA enriched organelles, such as the Membraneless Organelles (MLOs), nucleoli, found in the nuclei of mammalian cells. Therefore, PhenoVue 512 nucleic acid stain is the dye of choice for nucleoli staining.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue 512 nucleic acid stain	CP61	1 vial	5 mM (1.25 µmoles - 0.60 mg)	Solution in 250 µL 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 this product 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 vial.
- 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 512 nucleic acid stain	500 g/mol	Already reconstituted in 250 µL anhydrous DMSO to give a stock concentration of 5 mM (2.4 mg/mL)	0.5 µM - 10 µM (0.24 µg/mL - 4.8 µg/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 512 nucleic acid stain	3 µM (1.43 µg/L)	Approx. 15 to 45	Approx. 10 to 45	Approx. 20 to 70

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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 512 nucleic acid stain	512* 525**	590**	FITC, Cy3.5	nd***	70000	nd***

* In methanol ** In HBSS *** Not determined

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue 512 nucleic acid 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.

Note: PhenoVue 512 nucleic acid stain is compatible with live as well as fixed and permeabilized cells.

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 0.5-10 µM PhenoVue 512 nucleic acid stain for 30 min at RT.
6. **Washing:** Wash three times with HBSS for 5 min.
7. **Optional:** Incubate with 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.
8. **Washing:** Wash once with HBSS for 5 min.
9. Acquire images on an imaging device.

Live-cell imaging

1. Rinse briefly in HBSS.
2. Incubate with 0.5-10 µM PhenoVue 512 nucleic acid stain for 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

- When using PhenoVue 512 nucleic acid stain, avoid phosphate-based buffers as phosphate may compete with nucleic acid binding.
- PhenoVue 512 nucleic acid stain is cell-permeable. Therefore, this dye is compatible with live and fixed cells and permeabilization step is optional.
- When co-staining with PhenoVue 512 nucleic acid stain and PhenoVue Hoechst 33342 nuclear stain, optimization of the final concentration of PhenoVue Hoechst 33342 nuclear stain may be required as nuclear intensity staining decreases with increasing concentrations of PhenoVue 512 nucleic acid stain *(see Figure 4)*, presumably due to competitive binding to nucleic acid between the two dyes.
- Revvity PhenoVue 512 nucleic acid stain is comparable to SYTO™ 14.

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

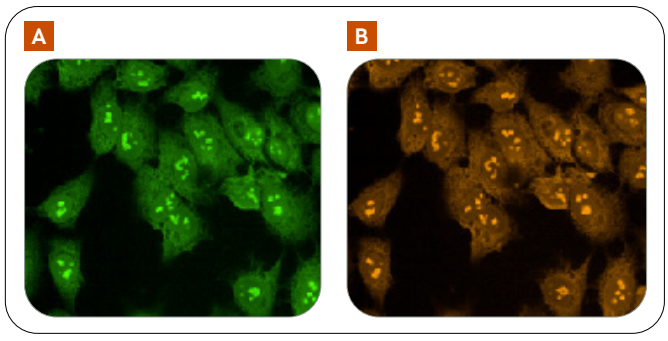

Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Fixed cells were stained with 3 µM of **PhenoVue 512 nucleic acid stain** for 30 min at RT. Images were acquired on the Operetta CLS™ high-content analysis system, using either FITC (A) or Cy3.5 (B) filter set.

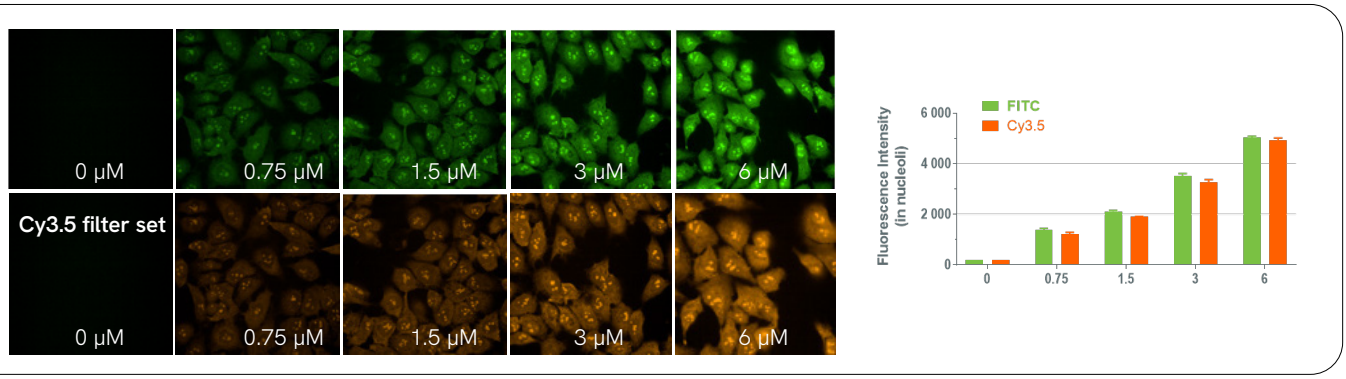

Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Fixed cells were stained with increasing concentrations of **PhenoVue 512 nucleic acid stain** for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system, using either FITC (A) or Cy3.5 (B) filter set. Image analysis was done by region mean intensity in the nucleoli.

Validation data

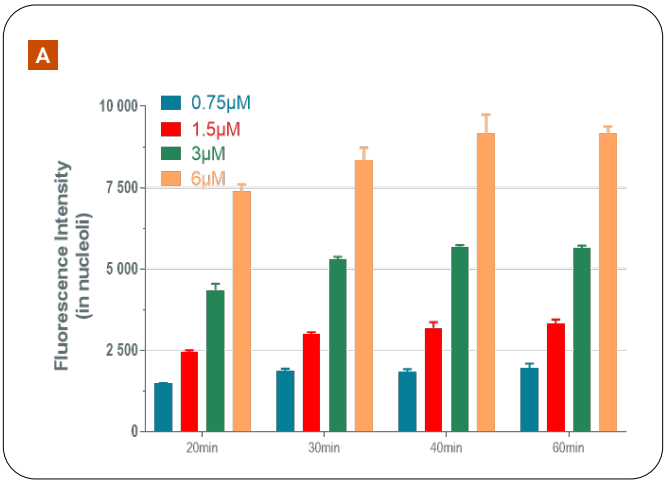

Figure 3A: 0ptimization of incubation time.

HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Fixed cells were stained with increasing concentrations of **PhenoVue 512 nucleic acid stain** for either 20, 30, 40 or 60 min at RT. Images were acquired on the Operetta CLS high-content analysis system, using Cy3.5 filter set. Image analysis was done by region mean intensity in the nucleoli.

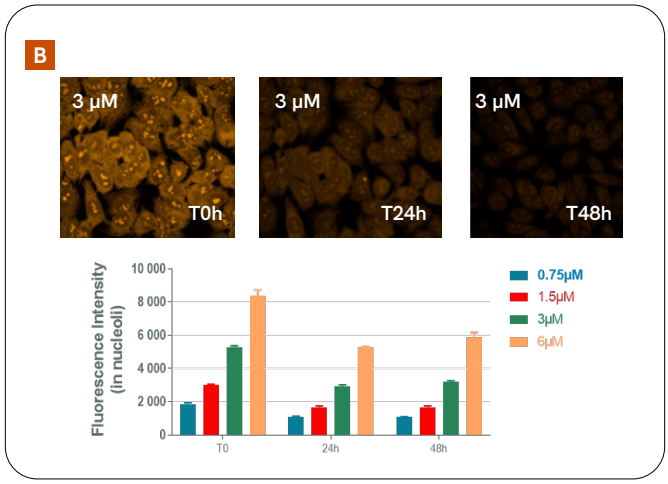

Figure 3B: Stability of the nucleoli signal over time.

HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Fixed cells were stained with increasing concentrations of **PhenoVue 512 nucleic acid stain** for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system, using Cy3.5 filter set immediately (T0), microplates were stored at 4 °C protected from light, then re-imaged at 24h and 48h. Image analysis was done by region mean intensity in the nucleoli. Note a signal decrease of PhenoVue 512 nucleic acid in the nucleoli which may be explained by a redistributed staining from RNA to DNA.

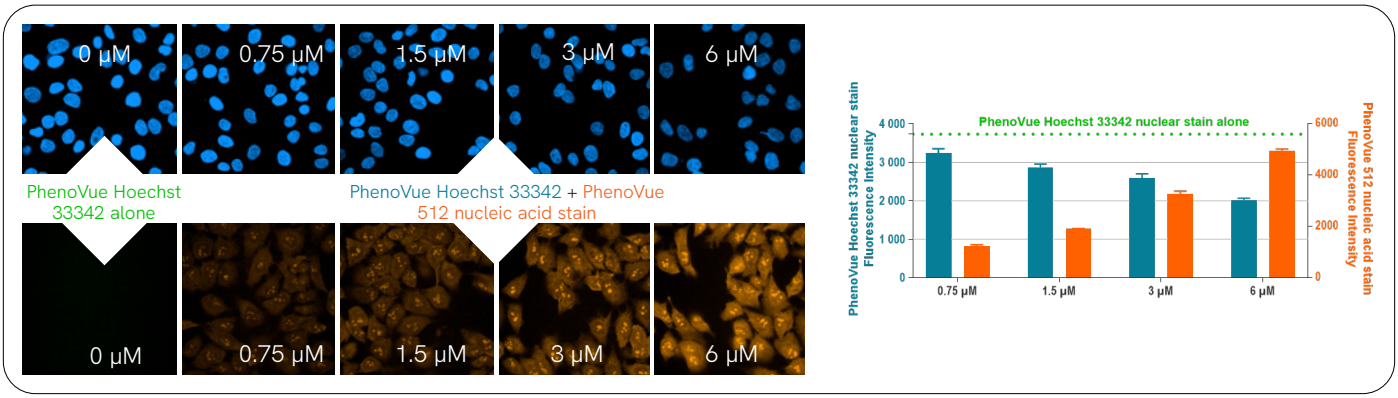

Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Fixed cells were stained with 2µg/mL of of **PhenoVue Hoechst 33342 nuclear stain** alone or in the presence of increasing concentrations of **PhenoVue 512 nucleic acid stain** for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system, using DAPI and FITC filter set. Image analysis was done by region mean intensity in the nucleoli. Note a dose-dependent decrease of PhenoVue Hoechst 33342 nuclear stain signal with PhenoVue 512 nucleic acid stain, which may be explained by competition between these two nucleic acid dyes.

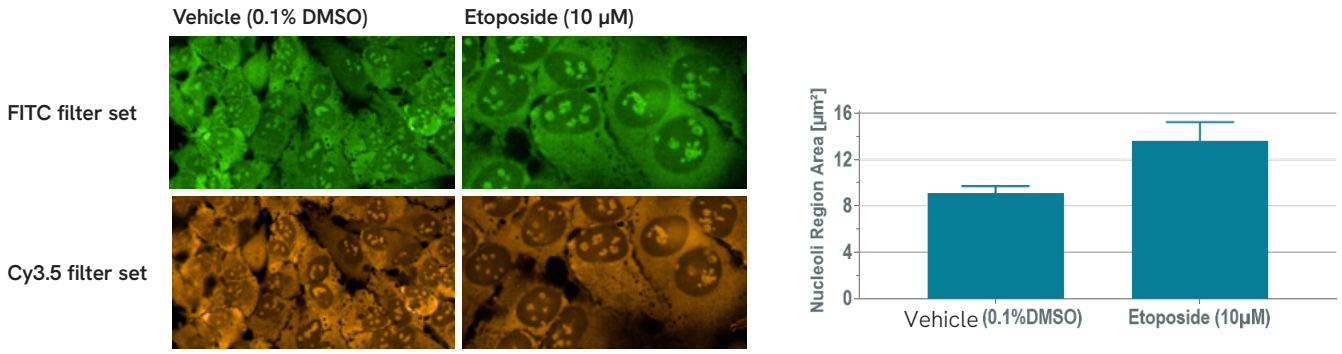

Figure 5: HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were treated with 10 µM etoposide, a topoisomerase II inhibitor which induces double stranded DNA breaks leading to G2 cell cycle arrest. After 48h treatment, cells were fixed and permeabilized then stained with 3 µM ***PhenoVue 512 nucleic acid stain*** for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system, using either FITC or Cy3.5 filters set. Image analysis was done by nucleoli region area. Note: G2 cell cycle arrest induced by Etoposide is associated with morphological changes such as size increase of cell, nucleus and nucleoli, as observed by Gustafsdottir et al. (PLoS One 2013), and Nyffeler et al. (Toxicol Appl Pharmacol 2020).

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com