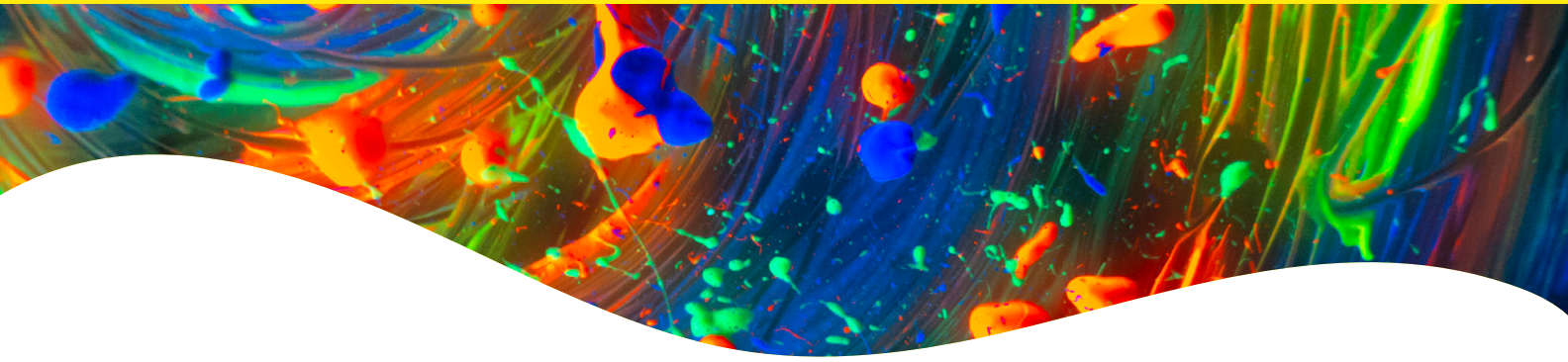
PhenoVue DNA Damage and Apoptosis Staining Kit (gH2AX, cleaved PARP) for 1x384



Overview

DNA is continuously exposed to endogenous and exogenous insults that can result in single-strand breaks (SSBs), double-strand breaks (DSBs), replication stress, or chemical modifications. If left unrepaired, these lesions can compromise genome integrity and may lead to genomic instability, senescence, or cell death. To counteract these threats, cells activate the DNA Damage Response (DDR), a coordinated signaling network that detects DNA lesions, promotes DNA repair, and contributes to cell fate decisions.

One of the earliest and most widely used markers of DNA double-strand breaks is the phosphorylation of histone H2AX at serine 139, generating γ-H2AX. Following DNA damage, γ-H2AX rapidly accumulates at sites of DNA lesions and supports the recruitment of key DDR proteins, including the MRE11-RAD50-NBS1 complex, MDC1, 53BP1, and BRCA1, leading to the formation of characteristic nuclear foci. Quantification of γ-H2AX is therefore commonly used to monitor DNA damage and evaluate the activity of DNA-damaging agents.

When DNA damage is severe or remains unrepaired, cells may activate apoptosis. During apoptosis, Poly (ADP-ribose) Polymerase 1 (PARP1), a key DNA repair enzyme, is cleaved by activated caspases, generating cleaved PARP1, a widely used marker of apoptotic pathway activation. The combined analysis of γ-H2AX and cleaved PARP1 can provide complementary information on DNA damage signaling and apoptosis-associated responses within fixed-cell imaging workflows.

The PhenoVue™ DNA Damage and Apoptosis Staining Kit provides a convenient solution for imaging DNA damage and apoptosis-associated markers in fixed cells. The kit combines PhenoVue™ Hoechst 33342 Nuclear Stain with antibodies targeting γ-H2AX and cleaved PARP1, enabling visualization of nuclear morphology together with DNA damage and apoptotic marker staining. With fluorescent labeling and compatibility with high-content imaging, the kit supports the characterization of compounds or conditions that induce DNA damage, activate DDR pathways, and/or trigger apoptosis-associated PARP1 cleavage.

Product information

Product name	Part no.	Number of vials per unit	Shipping conditions
PhenoVue DNA Damage and Apoptosis Staining Kit 1x384 (gH2AX, cleaved PARP1)	PDDAP11	8	Dry ice

Kit contents	Format	Packaging*	Storage
PhenoVue Hoechst 33342 Nuclear Stain	Liquid (H_2O)	1 vial of 70 µL (500X)	2-8 °C or below Protect from light
PhenoVue anti-Phospho H2AX (S139) – mouse IgG1 Antibody (100X)	Liquid	1 vial of 100 µL (100X)	-16 °C or below
PhenoVue anti-cleaved PARP1 Antibody (Rabbit)	Liquid	1 vial of 100 µL (100X)	-16 °C or below
PhenoVue Fluor 488 - Goat anti-Mouse antibody Highly Cross-Adsorbed (100X)	Liquid	1 vial of 100 µL (100X)	-16 °C or below Protect from light
PhenoVue Fluor 647 - Goat anti-Rabbit antibody Highly Cross-Adsorbed (100X)	Liquid	1 vial of 100 µL (100X)	-16 °C or below Protect from light
PhenoVue Paraformaldehyde, 4% Solution	Liquid	1 vial of 25 mL (1X)	2-8 °C or below Protect from light
PhenoVue Permeabilization, 0.5% Triton X-100 Solution	Liquid	1 vial of 25 mL (1X)	2-8 °C or below
PhenoVue dye diluent A (5X)	Liquid	1 vial of 8 mL (5X)	-16 °C or below

* Amount of reagents provided is sufficient for 1 x 384 well microplate using the concentrations.

Storage and stability

- After receiving, store PhenoVue Permeabilization 0.5% Triton X-100 solution and PhenoVue Paraformaldehyde, 4% Solution reagents at 2–8 °C, protected from light. Other reagents can be stored together at ≤ -16 °C or separately between ≤ -16 °C to 2-8 °C protected from light, as indicated in the table above. Avoid repeated freeze / thaw cycles.
- Allow the reagents to warm up to room temperature for 30 min before opening the vials and reconstituting. Aliquoted reagents must be stored at -16 °C or below and are stable for 6 months.
- After thawing, the PhenoVue dye diluent A (5x) may contain some aggregates which will not impair the product and image quality. If aggregate removal is preferred, the PhenoVue dye diluent A (5x) can be filtered (0.22 µm filter) prior to dilution. The diluted PhenoVue dye diluent A 1X must be stored at 2-8 °C for no more than 2 days.
- The stability of these products is supported until the expiration date provided in the certificate of analysis, when stored at recommended conditions and protected from light.

Safety information

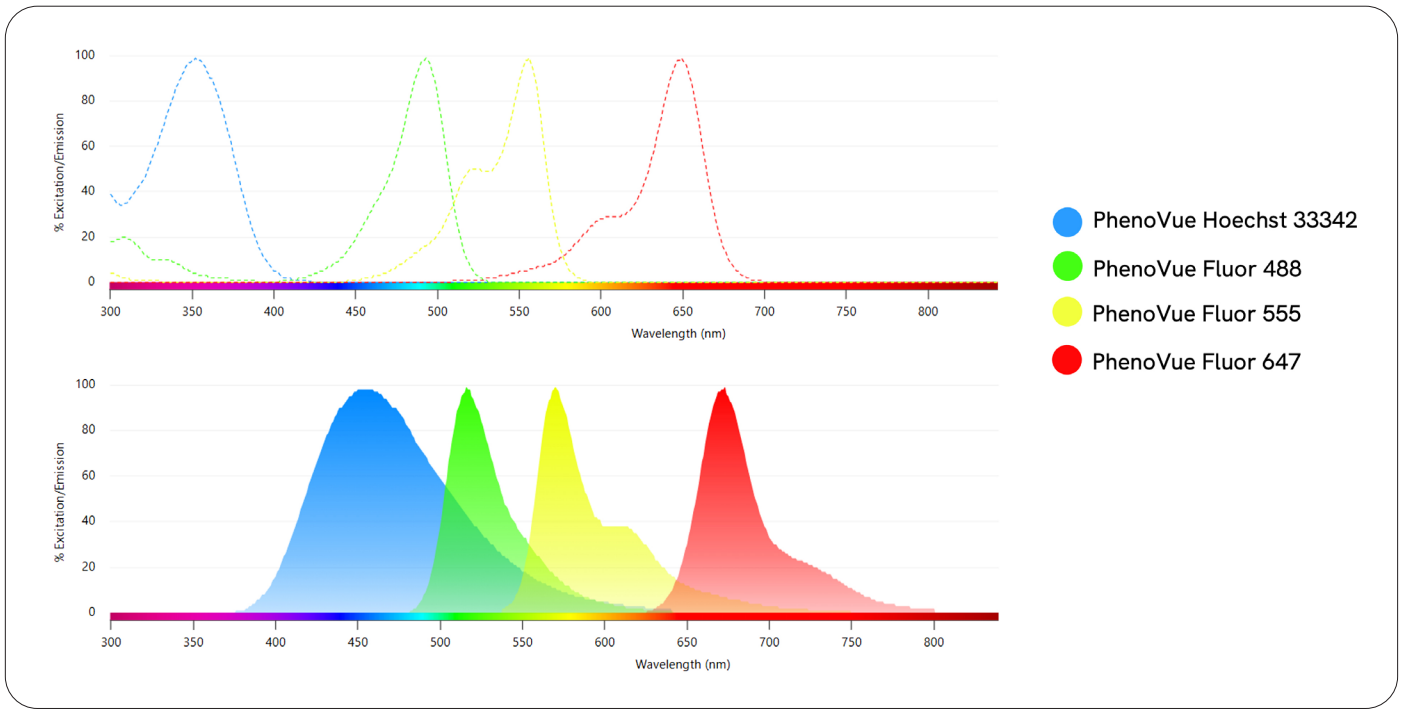
Chemical reagents are potentially harmful. Please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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 Hoechst 33342	357**	455**	DAPI	dsDNA: 0.38 ssDNA: 0.22	43,000	dsDNA: 16,340 ssDNA: 9,460
PhenoVue Fluor 488	495	520	FITC	92%	73,000	65,320
PhenoVue Fluor 555	555	570	Cy3	10%	155,000	15,500
PhenoVue Fluor 647	650	670	Cy5	30%	240,000	72,000

* In PBS pH 7.4 ** In methanol with 0.2 M HCl
Nd: not determined.

Please note that the PhenoVue Fluor 555 orange channel remains available for experimental flexibility, allowing users to incorporate additional markers tailored to their specific research needs.



Excitation and emission spectrum associated with the PhenoVue DNA Damage and Apoptosis Staining Kit components.

Other materials and reagents not provided

Reagents or consumables	Usage
PBS	Washing buffer
Distilled H_2O	Dilution of PhenoVue dye diluent A (5X)
PhenoPlate™ 384-well microplates*	Cell plating, stimulation, staining and imaging
Aluminum single-tab foil	Plate sealing to protect fluorescent probes from light

* This protocol can be adapted using PhenoPlate 96-well microplates. See Protocol Section for details. View our full range of high-quality imaging microplates at Revvity.com.

Reagent reconstitution and preparation of staining solutions

	Reagents	Preparation of staining solution
Buffers	PhenoVue Paraformaldehyde, 4% Solution	Ready to use
	PhenoVue Permeabilization 0.5% Triton X-100 Solution	Ready to use
	PhenoVue dye diluent A (5X)*	Dilute 5 times in distilled H_2O
Staining solution 1	PhenoVue anti-Phospho H2AX (S139) – mouse IgG1 Antibody (100X)	Dilute 100 times in diluent A (1x)
	PhenoVue anti-cleaved PARP1 Antibody (Rabbit)	Dilute 100 times in diluent A (1x)
Staining solution 2	PhenoVue Hoechst 33342 Nuclear Stain	Dilute 500 times in diluent A (1x)
	PhenoVue Fluor 488 - Goat anti-Mouse antibody Highly Cross-Adsorbed (100X)	Dilute 100 times in diluent A (1x)
	PhenoVue Fluor 647 - Goat anti-Rabbit antibody Highly Cross-Adsorbed (100X)	Dilute 100 times in diluent A (1x)

* PhenoVue dye diluent A 1x is used as staining solution diluent and as saturation buffer.

Preparation of buffers and staining solutions

- **Prepare stock solutions of buffers and diluent**
  - Fixative solution: PhenoVue Paraformaldehyde 4% Solution is provided ready-to-use.
  - Permeabilization solution: PhenoVue Permeabilization 0.5% Triton X-100 Solution is provided ready-to-use.
  - Saturation and staining diluent solution: Prepare PhenoVue Dye Diluent A (1x) by diluting the supplied PhenoVue Dye Diluent A (5x) five-fold in distilled water.
- **Prepare staining solutions in PhenoVue Dye Diluent A (1x)**

  Protect stock reagents and staining solutions from light.
  - Staining Solution 1

    PhenoVue anti-Phospho H2AX (S139) Mouse IgG1 Antibody (100x): dilute 1:100 in PhenoVue Dye Diluent A (1x).

    PhenoVue anti-cleaved PARP1 Rabbit Antibody (100x): dilute 1:100 in PhenoVue Dye Diluent A (1x).
  - Staining Solution 2

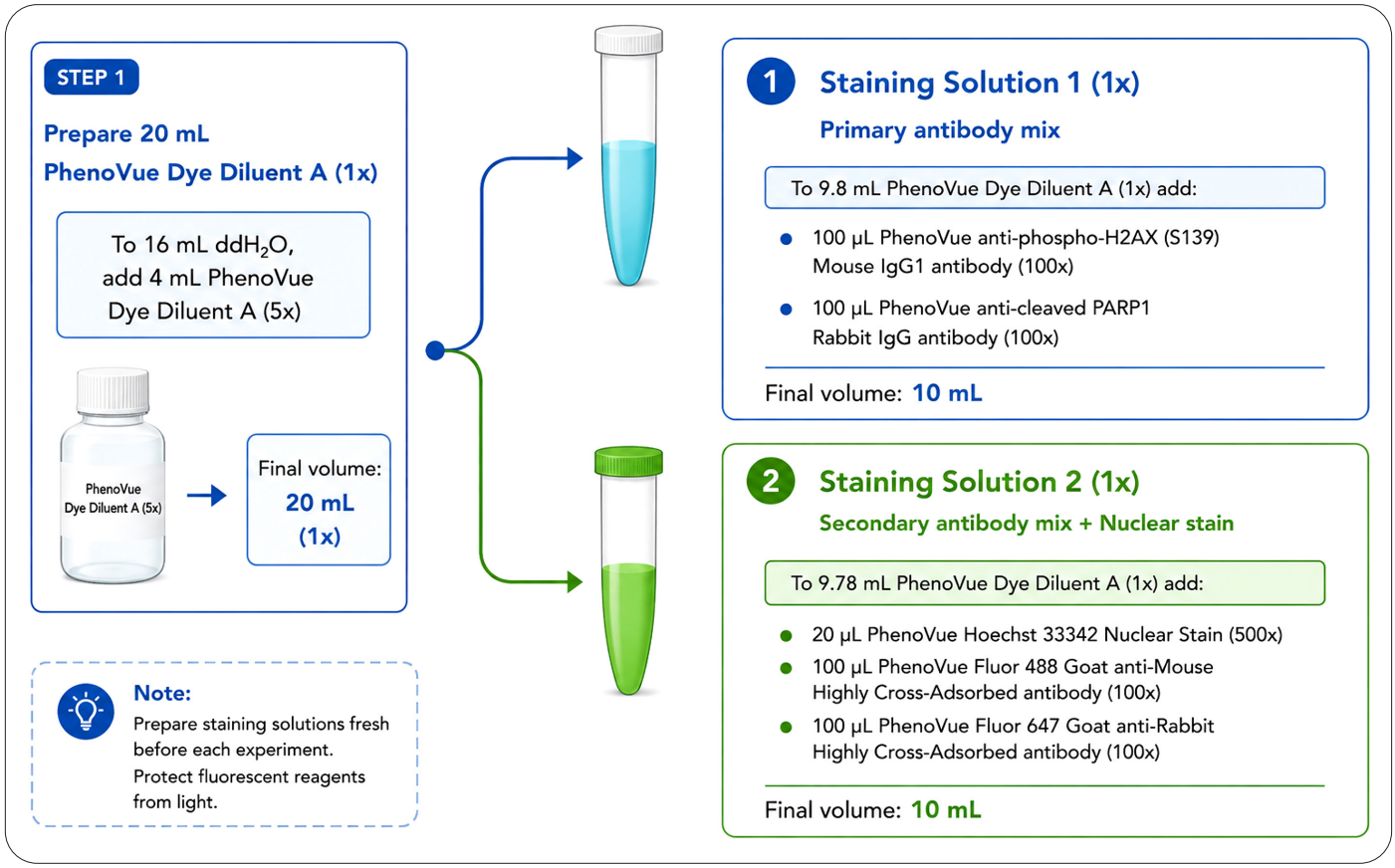
    PhenoVue Hoechst 33342 Nuclear Stain (500x): dilute 1:500 in PhenoVue Dye Diluent A (1x).

    PhenoVue Fluor 488 Goat anti-Mouse Highly Cross-Adsorbed Antibody (100x): dilute 1:100 in PhenoVue Dye Diluent A (1x).

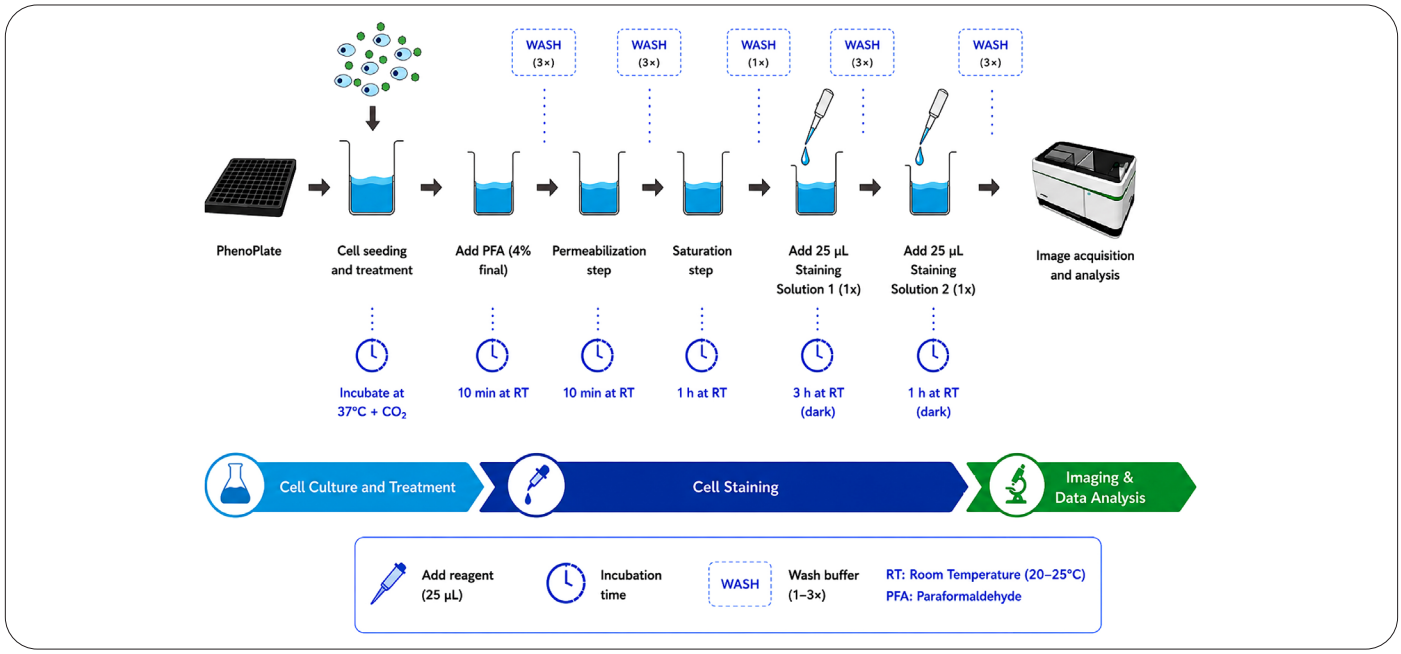
    PhenoVue Fluor 647 Goat anti-Rabbit Highly Cross-Adsorbed Antibody (100x): dilute 1:100 in PhenoVue Dye Diluent A (1x).

Example preparation of staining solutions

The following example describes the preparation of 10 mL staining solution 1 and 10 mL of staining solution 2, sufficient for 1 x 384-well plate (or 2 x 96-well plates).



Experimental workflow



* This protocol can be adapted using PhenoPlate 96-well microplates. See Protocol Section for details.

Protocol for 384-well imaging plates

This protocol can be adapted to 96-well PhenoPlate microplates by adjusting cell seeding density and reagent volumes.

Rinse briefly in phosphate-buffered saline (PBS) then proceed with cell fixation.

1. **Fixation:** Add 25 µL ready-to-use PhenoVue paraformaldehyde 4% solution for 10 min at room temperature.
2. **Washing:** Wash three times with PBS.
3. **Permeabilization:** Add 25 µL ready-to-use PhenoVue permeabilization solution for 10 min at room temperature.
4. **Washing:** Wash three times with PBS for 5 min.
5. **Saturation:** Incubate with 25 µL PhenoVue dye diluent A 1x for 1h at room temperature.
6. **Add 25 µL of Staining Solution 1.**
7. **Incubate for 3h** at room temperature, protected from light.
8. **Washing:** Wash three times with PBS for 5 min.
9. **Add 25 µL of Staining Solution 2.**
10. **Incubate for 1h** at room temperature, protected from light.
11. **Washing:** Wash three times with PBS for 5 min.
    Note: HBSS can be supplemented with 0.05% sodium azide if image acquisition is not performed immediately.
12. **Seal the plate** (optional) and store at 4 °C protected from light until image acquisition.
13. **Image acquisition:** Refer to the following section for recommended microscope settings
14. **Image Analysis.**

Tips and guidance

- **Enhanced reagent concentrations**

Reagent concentrations have been carefully selected to reduce fluorescence crosstalk on Revvity high-content imaging systems. Increasing reagent concentrations may result in increased channel crosstalk and reduced image quality.

- **Addition of a third target using the PhenoVue Fluor 555 channel**

For added flexibility, the PhenoVue Fluor 555 channel remains available for the detection of additional proteins or cellular events using compatible fluorescent probes or antibody-based staining reagents. Additional targets may be incorporated using a primary antibody raised in a species other than mouse or rabbit, together with a compatible PhenoVue Fluor 555 Highly Cross-Adsorbed secondary antibody. For example, PhenoVue Fluor 555 Goat anti-Rat Highly Cross-Adsorbed (part no. 2GXRT555H1) can be used with rat primary antibodies while preserving sufficient spectral separation from the γ-H2AX and cleaved PARP1 detection channels.

- **Species compatibility**
  - PhenoVue primary antibodies (unconjugated): The anti-Phospho H2AX (S139) and anti-cleaved PARP1 antibodies have been characterized on human cellular models and may be compatible with mouse cellular models, according to sequence homology. The PhenoVue anti-Phospho H2AX (S139) antibody is a mouse IgG1, whereas the PhenoVue anti-cleaved PARP1 antibody is a rabbit IgG.
  - PhenoVue Fluor secondary antibodies: PhenoVue Fluor 488 Goat anti-Mouse secondary antibody highly cross-adsorbed is specific for mouse immunoglobulins. However, it is not isotype-specific and may recognize multiple mouse IgG subclasses, including IgG1, IgG2a, IgG2b, IgG2c, and IgG3.

PhenoVue Fluor 647 Goat anti-Rabbit secondary antibody highly cross-adsorbed specifically detects rabbit primary antibodies.

- **Improved staining consistency in 384-well microplates**

When using 384-well microplates, centrifugation of the plate at 500 × g for 1 minute at room temperature between staining steps may facilitate reagent settling at the bottom of the wells and improve staining consistency.

Guidance on acquisition settings

The PhenoVue DNA Damage and Apoptosis Staining Kit allows for multiplexing with 3 to 4 colors simultaneously. For improved fluorescent signal and image quality on a Revvity high-content screening system, we provide the following acquisition settings for reference, depending on the instrument used:

HCS instruments		PhenoVue Hoechst 33342	PhenoVue Fluor 488	PhenoVue Fluor 555	PhenoVue Fluor 647
Opera Phenix™ Plus 5 lasers	Excitation laser (nm)	375	488	561	640
	Emission filters (nm)	435-480	500-550	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation laser (nm)	405	488	561	640
	Emission filters (nm)	435-480	500-550	570-630	650-760
Operetta CLS 4 or 8 LED – 1600 & 1601	Excitation LED (filters) (nm)	370 (355-385)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filters (nm)	430-500	500-550	570-650	655-760

Applications

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

Assay validation

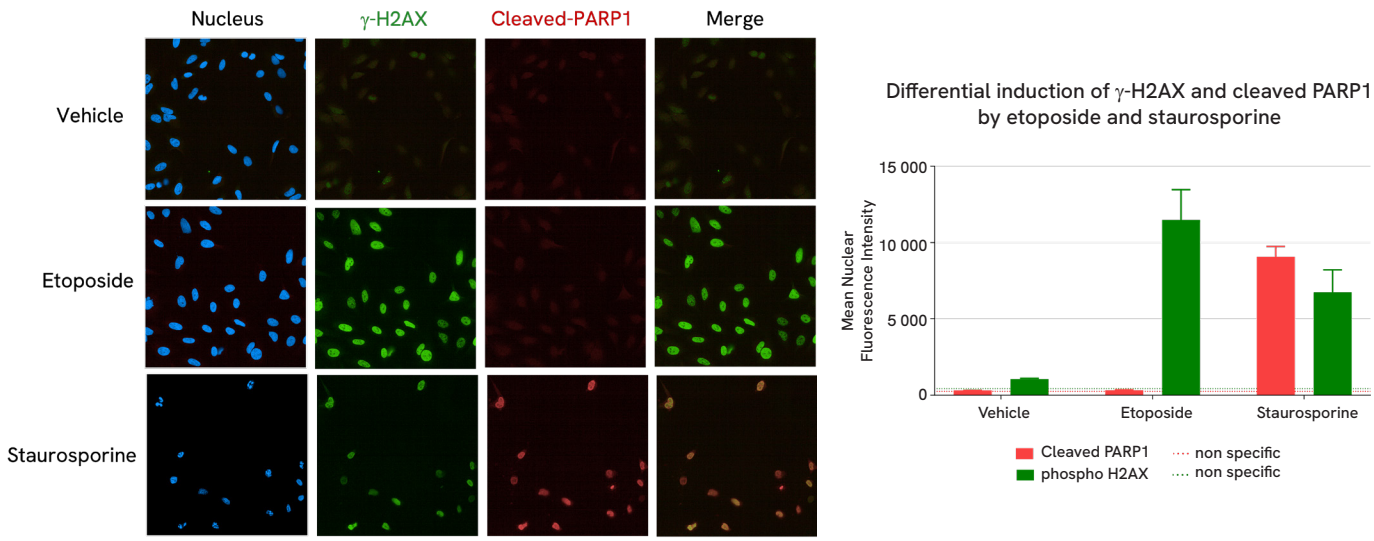


Figure 1: **Simultaneous detection of DNA damage and apoptosis using the PhenoVue™ DNA Damage & Apoptosis Staining Kit.**
HeLa cells were seeded in PDL-coated PhenoPlate 96-well microplates at a density of 10,000 cells per well and incubated overnight at 37 °C in 5% CO_2. Cells were subsequently treated for 4 h with either etoposide (50 µM), staurosporine (1.25 µM), or vehicle control (0.5% DMSO). Following fixation, permeabilization, and blocking, cells were stained using the PhenoVue DNA Damage & Apoptosis Staining Kit according to the provided protocol (3 h incubation with primary antibodies followed by 1 h incubation with secondary antibodies and Hoechst 33342 at room temperature). Images were acquired on an Operetta CLS High-Content Analysis System using a 40× water-immersion objective in confocal mode.
Vehicle-treated cells showed only low basal levels of phospho-H2AX (γ-H2AX) and cleaved PARP1 staining. Etoposide treatment induced a marked increase in γ-H2AX signal with minimal cleaved PARP1 induction, consistent with activation of the DNA damage response. In contrast, staurosporine treatment strongly increased cleaved PARP1 staining and was accompanied by elevated γ-H2AX levels, consistent with apoptosis-associated DNA fragmentation. These results are consistent with the simultaneous detection and differentiation of DNA damage and apoptotic responses within the same sample using the PhenoVue DNA Damage & Apoptosis Staining Kit.

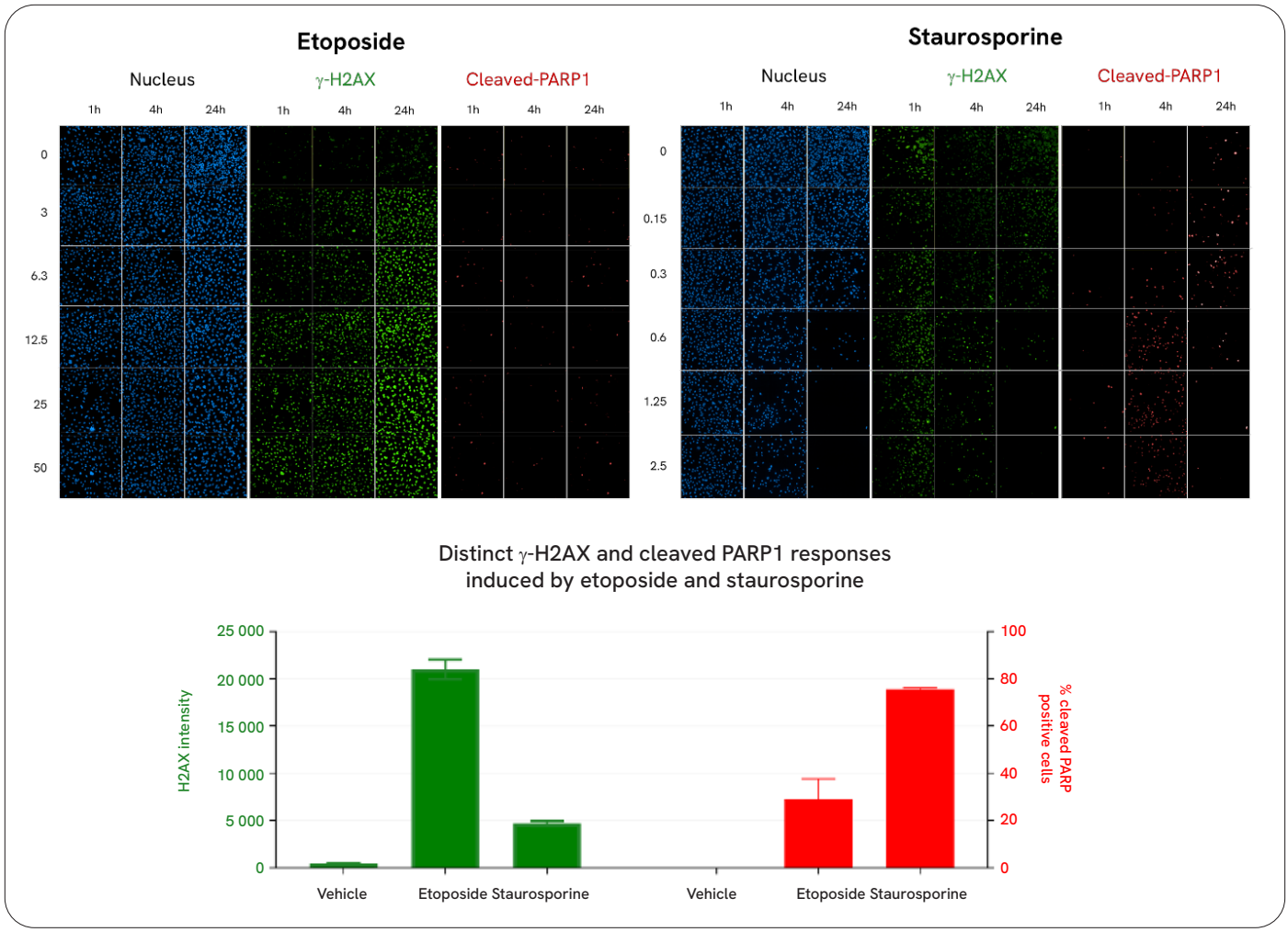


Figure 2: **Distinct γ-H2AX and cleaved PARP1 responses induced by etoposide and staurosporine.**
HeLa cells were seeded in PDL-coated PhenoPlate 96-well microplates at a density of 10,000 cells per well and incubated overnight at 37 °C in 5% CO_2. Cells were then treated with increasing concentrations of either etoposide or staurosporine for 1, 4, or 24 h. Following fixation, permeabilization, and blocking, cells were stained using the PhenoVue™ DNA Damage & Apoptosis Staining Kit according to the provided protocol (3 h incubation with primary antibodies followed by 1 h incubation with secondary antibodies and Hoechst 33342 at room temperature). Images were acquired on an Operetta CLS High-Content Analysis System using a 20× water-immersion objective in confocal mode.

Representative images illustrate the dose- and time-dependent induction of γ-H2AX and cleaved PARP1 following treatment with etoposide or staurosporine. Etoposide primarily induced γ-H2AX staining, consistent with activation of the DNA damage response, whereas staurosporine strongly increased the proportion of cleaved PARP1-positive cells, indicative of apoptosis induction. The summary graph highlights representative conditions corresponding to maximal biomarker induction, namely 50 µM etoposide for 24 h and 1.25 µM staurosporine for 4 h. Under these conditions, etoposide elicited a robust γ-H2AX response with limited apoptosis, whereas staurosporine induced a strong apoptotic response accompanied by a more moderate increase in γ-H2AX. These results are consistent with the ability to distinguish DNA damage and apoptotic responses within the same cellular model using the PhenoVue™ DNA Damage & Apoptosis Staining Kit.