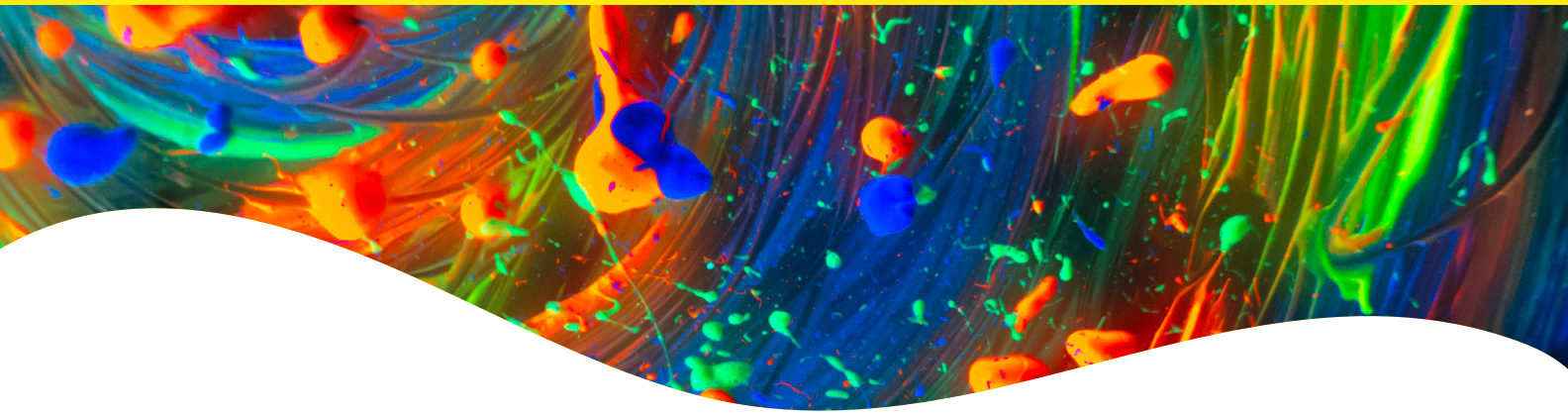
PhenoVue anti-PARP1 – mouse IgG2a antibody
PhenoVue anti-PARP1 – rabbit IgG antibody



Overview

DNA damage spans a wide spectrum, from single-strand breaks (SSBs) and double-strand breaks (DSBs) to complex base modifications. To preserve genomic integrity, cells activate the DNA damage response (DDR), a coordinated network of detection and repair pathways. This process is central not only to understanding genome stability but also to evaluating cytotoxic agents and developing new anticancer therapies.

PARP1 (Poly (ADP-ribose) Polymerase 1) is a key nuclear enzyme that senses SSBs and other DNA lesions. Upon recruitment to sites of damage, PARP1 catalyzes PARylation, recruits DNA repair factors, and remodels chromatin to facilitate repair. The therapeutic relevance of PARP1 was established with the approval of PARP inhibitors (PARPi), which exploit the principle of synthetic lethality. Tumors with defects in homologous recombination repair pathways, such as those carrying BRCA1 or BRCA2 mutations, are particularly sensitive to PARPi (Lord & Ashworth, Science, 2018). Beyond conventional inhibition, new approaches now aim to suppress PARP1 expression through targeted protein degradation (TPD), including PROTAC degraders (Cao et al., *J. Med. Chem.*, 2020). These advances highlight the importance of accurately detecting and quantifying PARP1 across cellular models.

The **PhenoVue™ anti-PARP1 mouse IgG2a** and **PhenoVue anti-PARP1 rabbit IgG antibodies** are designed to support imaging and high-content analysis workflows. Both are compatible with PhenoVue Fluor goat anti-mouse or anti-rabbit highly cross-adsorbed antibodies for multiplex fluorescent detection. In addition, the rabbit anti-PARP1 antibody is compatible with the PhenoVue DNA damage response staining kit (part no. PDDR11), enabling seamless multiplexing with PhenoVue Fluor 555 goat anti-rabbit highly cross-adsorbed antibody (part no. 2GXRB555H1).

Product information

Product name	PhenoVue anti-PARP1 – mouse IgG2a antibody	PhenoVue anti-PARP1 – rabbit IgG antibody
Part number	PAMSPARP11	PARBPARP11
Packaging	1 vial of 100 µL	1 vial of 100 µL
Concentration	100x	100x
Format	Liquid	Liquid
Clonality	Monoclonal	Monoclonal
Host species	Mouse	Rabbit
Isotype	IgG2a, κ	IgG, κ
Species-reactivity	Human verified	Human verified
Immunogen	The exact immunogen used to generate this antibody is proprietary information.	The exact immunogen used to generate this antibody is proprietary information.
Purification	Affinity chromatography	Affinity chromatography
Formulation	PO_4 pH7 100 mM – 0.1% BSA	PO_4 pH7 100 mM – 0.1% BSA
Applications	High-content imaging, immunofluorescence, cell imaging, microscopy	High-content imaging, immunofluorescence, cell imaging, microscopy
Shipping conditions	Dry ice	Dry ice
Storage conditions	-16 °C or below.	-16 °C or below.

Stability

- The stability of this product is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended.
- To avoid multiple freeze/thaw cycles, freeze the stock solution in aliquots.

Protocols

Cell culture

Seed cells in PhenoPlate™ 384-well 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.

Fixed-cell imaging protocol

1. **Rinse:** Briefly rinse the cells with phosphate-buffered saline (PBS) before proceeding with fixation.
2. **Fixation:** Add ready-to-use PhenoVue paraformaldehyde 4% methanol-free solution (PVPFA41) for 10-20 min at room temperature.
3. **Washing:** Wash three times with PBS.
4. **Permeabilization:** Add PhenoVue permeabilization solution (PVPERM051) diluted to 0.1% Triton X-100 in PBS, for 10 min at room temperature.
5. **Washing:** Wash three times with PBS.
6. **Saturation:** Incubate with PhenoVue dye diluent A (PVDDA1), diluted at 1x in H_2O for 1 h at room temperature to block non-specific binding.
7. **Washing:** Wash three times with PBS.
8. **Primary antibody incubation:** Add 25 µL per well of either the anti-PARP1 mouse IgG2a antibody (1x) or the anti-PARP1 rabbit IgG antibody (1x), diluted in PhenoVue dye diluent A (1x). Incubate for 3 hours at room temperature.
9. **Washing:** Wash three times with PBS.
10. **Fluorescent secondary antibody incubation:** Add 25 µL per well of the appropriate secondary antibody (PhenoVue Fluor goat anti-mouse highly cross-adsorbed antibody or PhenoVue Fluor goat anti-rabbit highly cross-adsorbed antibody), matching the species of the primary antibody. Dilute the secondary antibody in PhenoVue dye diluent A (1x) to a final concentration of 5-10 µg/mL. Incubate for 1 hour at room temperature, protected from light.

Optional step

At this stage, additional reagents such as PhenoVue Hoechst 33342 nuclear stain (1–2 µg/mL) or PhenoVue Fluor 555 phalloidin (10–50 nM) may be added to the same preparation mix for nuclear or cytoskeletal counterstaining.

11. **Washing:** Wash three times with PBS.
12. **Imaging:** Acquire images using an imaging device of your choice.

* PhenoPlate 96-well microplates may also be used. Adjust the cell density accordingly and increase the corresponding volumes by 2-fold.

Tips

When multiplexing the PhenoVue PARP1 IgG antibodies with primary antibodies from other species, it is strongly recommended to use PhenoVue anti-species highly cross-adsorbed secondary antibodies. This minimizes the risk of cross-reactivity and ensures specific signal detection.

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

Assay validation

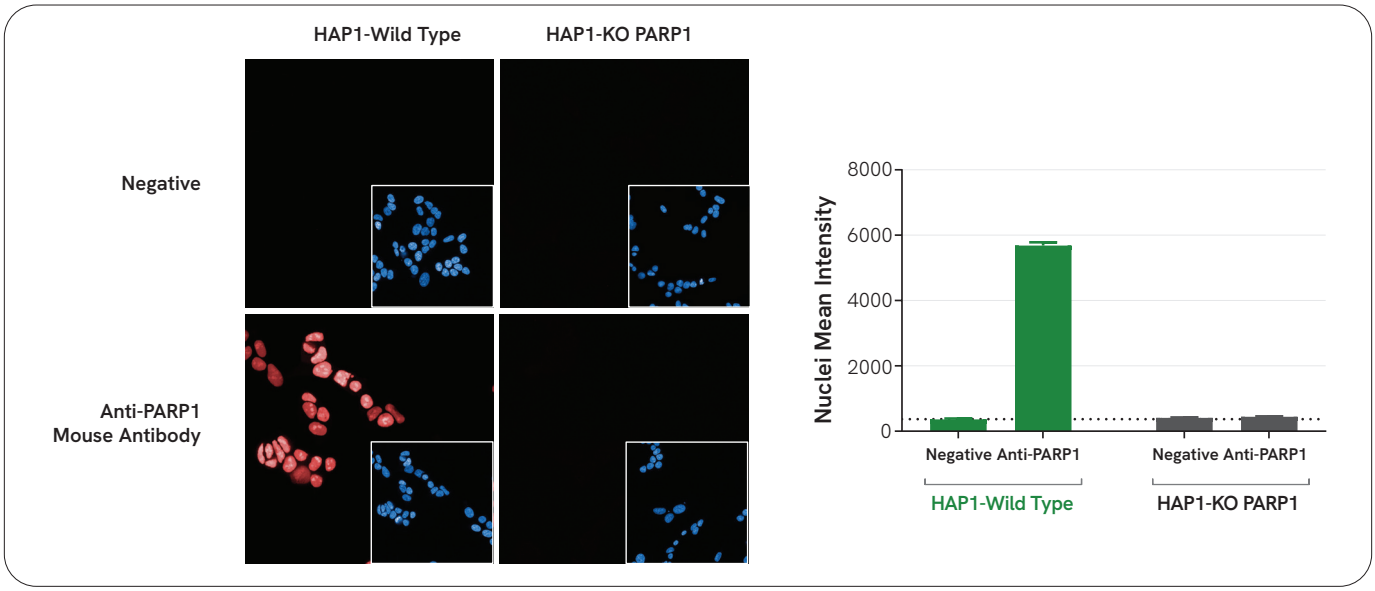


Figure 1: High specificity of the primary anti-PARP1 antibody.

Figure 1 shows HAP1 wild-type (WT) and HAP1 PARP1-knockout (KO) cells (Revvity) seeded in PhenoPlate 96-well microplates at a density of 16,000 cells per well and incubated overnight at 37 °C with 5% CO_2. After fixation, permeabilization, and blocking, cells were stained with the PhenoVue anti-PARP1 mouse IgG2a primary antibody (1x, 3 h, RT), followed by the PhenoVue Fluor 647 goat anti-mouse highly cross-adsorbed secondary antibody (10 µg/mL, 1 h, RT) and PhenoVue Hoechst 33342 nuclear stain (2 µg/mL). Images were acquired on the Operetta CLS™ high-content analysis system using a 63x water-immersion objective in confocal mode. A strong nuclear signal was observed in wild-type cells, while no staining was detected in PARP1-KO cells, confirming the target specificity of the primary antibody. Comparable results (not shown) were also obtained with the PhenoVue anti-PARP1 rabbit IgG antibody.

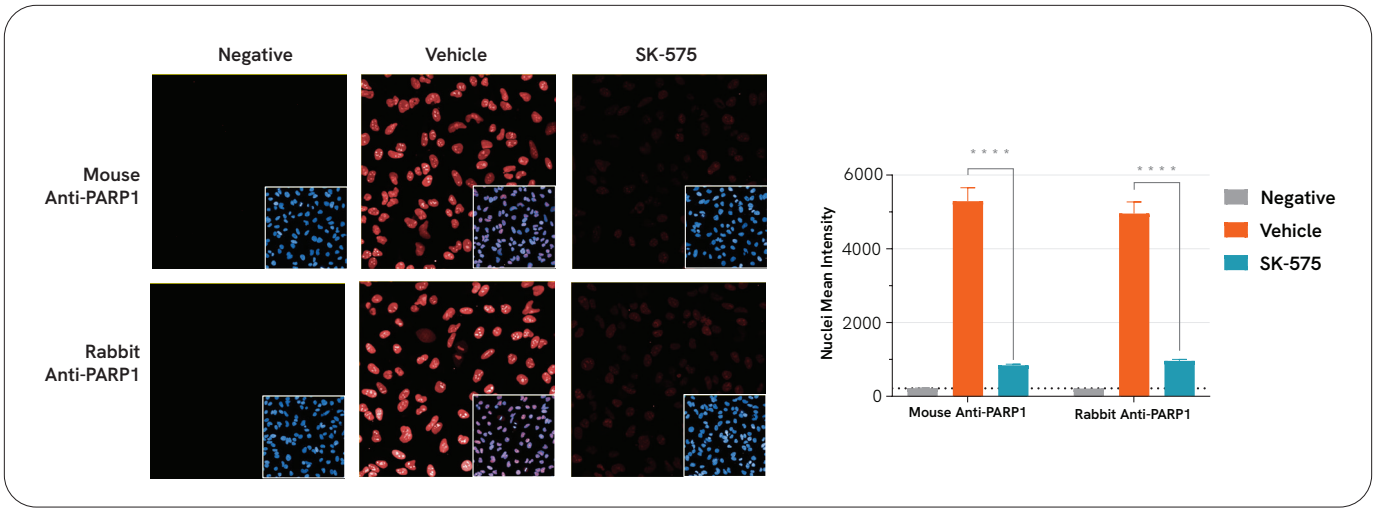


Figure 2: Monitoring targeted protein degradation of PARP1 with SK-575 PROTAC compound.

Figure 2 shows HeLa cells seeded in PhenoPlate 96-well microplates at a density of 10,000 cells per well and incubated overnight at 37 °C with 5% CO_2. Cells were then treated for 24 hours with either SK-575 compound (100 nM in culture medium) or vehicle control (0.01% DMSO). After fixation, permeabilization and blocking, cells were stained with the PhenoVue anti-PARP1 mouse IgG2a primary antibody (1x, 3 h, RT; upper panel) or the PhenoVue anti-PARP1 rabbit IgG primary antibody (1x, 3 h, RT; lower panel). Secondary detection was performed with PhenoVue Fluor 647 goat anti-mouse highly cross-adsorbed antibody (10 µg/mL, 1 h, RT; upper panel) or PhenoVue Fluor 647 goat anti-rabbit highly cross-adsorbed antibody (10 µg/mL, 1 h, RT; lower panel), together with PhenoVue Hoechst 33342 nuclear stain (2 µg/mL). Images were acquired using the Operetta CLS high-content analysis system with a 40x water-immersion objective in confocal mode. Treatment with SK-575 resulted in a strong reduction of PARP1 expression (–84% to –88%), consistent with published reports (Cao et al., *J. Med. Chem.*, 2020).

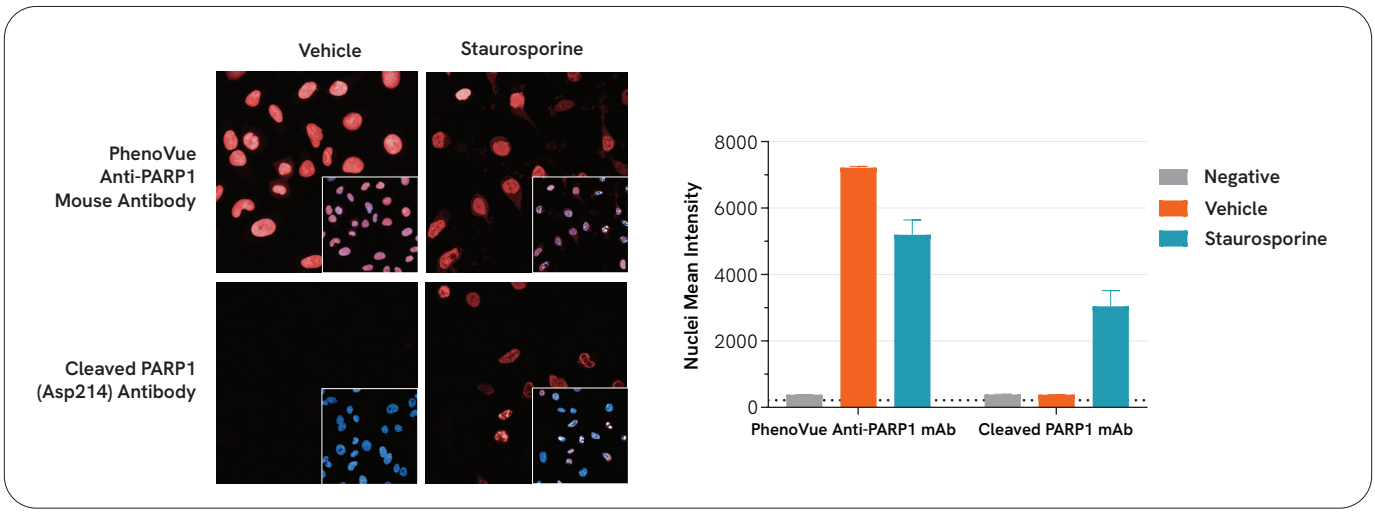


Figure 3: PhenoVue anti-PARP1 antibody detects both full-length and cleaved-PARP1 (Asp214) forms.

Figure 3 shows HeLa cells seeded in PhenoPlate 96-well microplates at a density of 10,000 cells per well and incubated overnight at 37 °C with 5% CO_2. Cells were treated for 4 hours with either vehicle control (0.3% DMSO) or staurosporine (3 µM). After fixation, permeabilization and blocking, cells were stained with either the PhenoVue anti-PARP1 mouse IgG2a primary antibody (upper panel) or an anti-cleaved-PARP1 (Asp214) rabbit IgG primary antibody (lower panel). Staining was followed by incubation with the corresponding PhenoVue Fluor 647 goat anti-mouse or goat anti-rabbit highly cross-adsorbed secondary antibody (10 µg/mL, 1 h, RT) together with PhenoVue Hoechst 33342 nuclear stain (2 µg/mL). Images were acquired using the Operetta CLS high-content analysis system with a 63x water-immersion objective in confocal mode. As expected, the reference anti-cleaved-PARP1 antibody detected PARP1 cleavage (Asp214) in staurosporine-treated cells. The PhenoVue anti-PARP1 antibody labeled both full-length PARP1 in vehicle-treated cells and cleaved PARP1 in staurosporine-treated cells, confirming recognition of both forms.

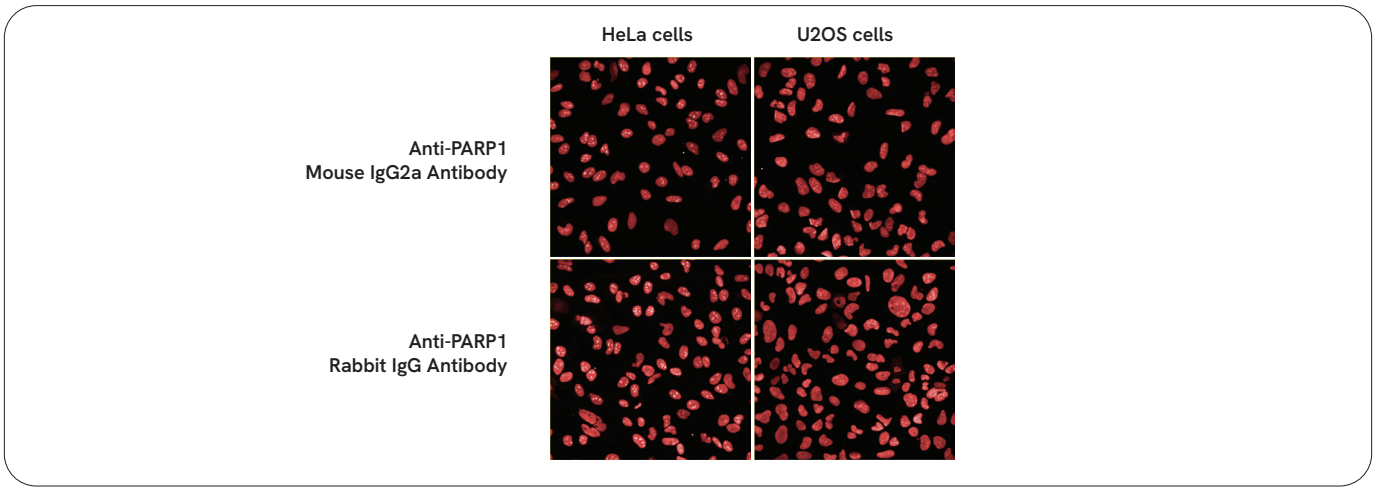


Figure 4: Validation of PhenoVue anti-PARP1 antibodies across multiple cell models.

Figure 4 shows HeLa and U2OS cells seeded in PhenoPlate 96-well microplates and incubated overnight at 37 °C with 5% CO_2. After fixation, permeabilization, and blocking, cells were stained with either the PhenoVue anti-PARP1 mouse IgG2a or the PhenoVue anti-PARP1 rabbit IgG primary antibody (1x, 3 h, RT). Secondary detection was performed with PhenoVue Fluor 647 goat anti-mouse or anti-rabbit highly cross-adsorbed antibodies (10 µg/mL, 1 h, RT). Images were acquired using the Operetta CLS high-content analysis system with a 40x water-immersion objective, in confocal mode. Across all three models, the PhenoVue anti-PARP1 antibodies produced strong and specific nuclear staining of PARP1.

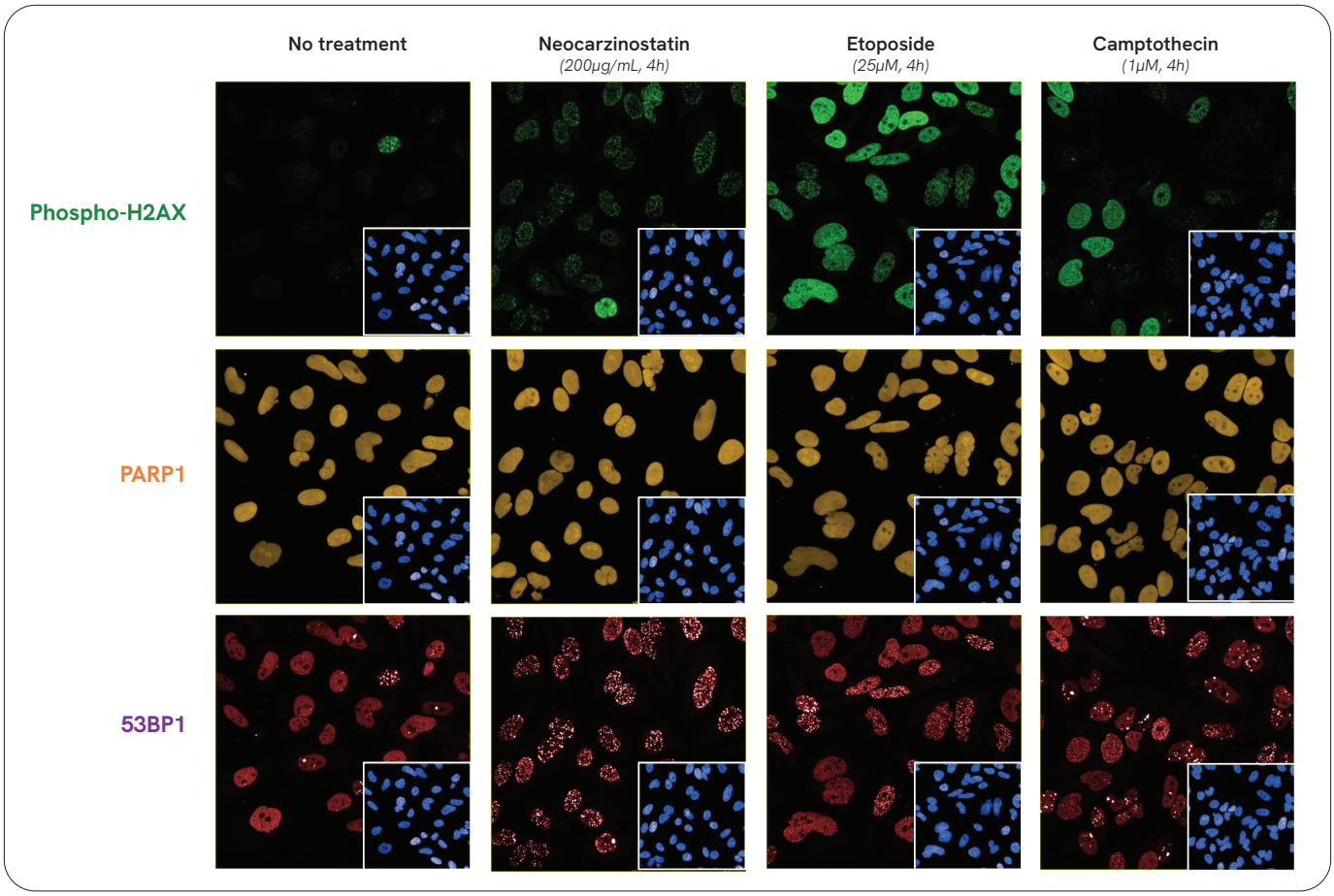


Figure 5: Multiplex detection of PARP1 with the PhenoVue DNA damage response staining kit.

Figure 5 shows HeLa cells seeded in PhenoPlate 96-well microplates at a density of 10,000 cells per well and incubated overnight at 37 °C with 5% CO_2. Cells were then treated for 4 hours with neocarzinostatin (200 µg/mL), etoposide (25 µM), or camptothecin (1 µM), or left untreated. Following fixation, permeabilization, and blocking, cells were stained using the PhenoVue DNA damage response staining kit (PDDR11) with the addition of the PhenoVue anti-PARP1 rabbit IgG antibody (1x). The primary antibody mix included anti-phospho-H2AX mouse IgG and anti-53BP1 rat IgG. Detection was performed using PhenoVue Fluor 555 goat anti-rabbit highly cross-adsorbed antibody (2GXRB555H1, 10 µg/mL) together with appropriate anti-mouse secondary antibodies. Images were acquired with the Opera Phenix™ Plus high-content screening system using a 63x water-immersion objective in confocal mode, with several z-planes captured and displayed as maximum projections. In line with published findings, DNA-damaging compounds increased γH2AX and 53BP1 foci without altering PARP1 expression or localization.