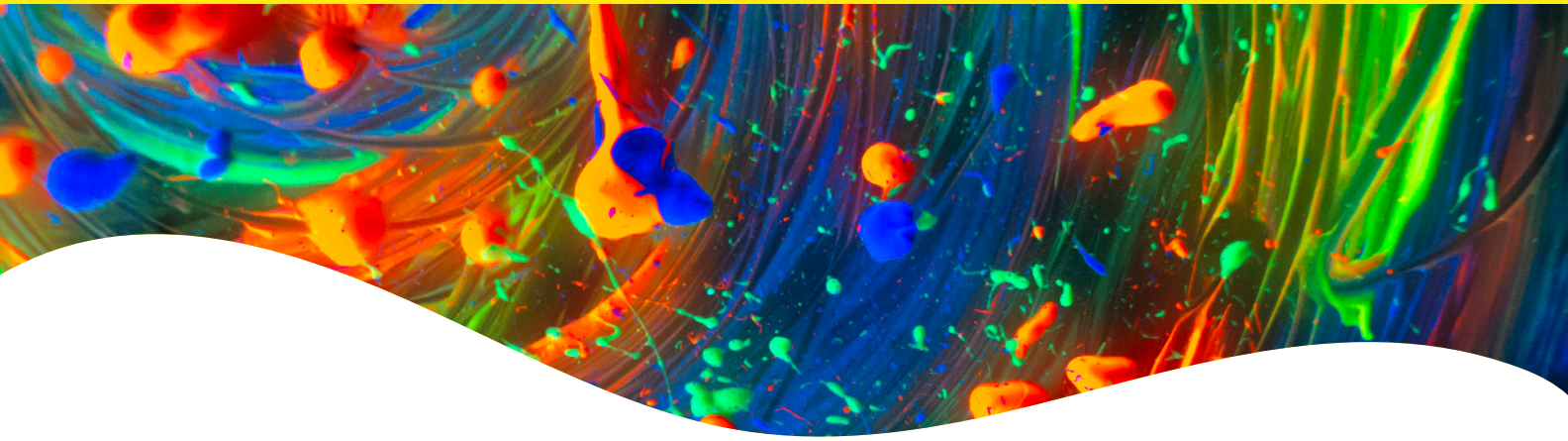
PhenoVue anti-cleaved PARP1 Antibodies (Mouse IgG1 and Rabbit IgG)



Overview

Apoptosis is a tightly regulated form of programmed cell death that is central to tissue homeostasis, development, immune regulation, and the cellular response to toxic or therapeutic stress. Reliable detection of apoptotic cells is therefore important for phenotypic profiling, compound characterization, mechanism-of-action studies, and high-content analysis workflows.

PARP1 is a nuclear enzyme involved in DNA damage sensing and repair. During apoptosis, activated executioner caspases cleave PARP1, generating a characteristic cleaved PARP1 fragment that is widely used as a marker of caspase-dependent apoptosis. Because the cleaved form accumulates in apoptotic cells, immunostaining for cleaved PARP1 provides a direct cellular readout of apoptotic pathway activation.

The PhenoVue™ anti-cleaved PARP1 mouse IgG1 antibody and PhenoVue anti-cleaved PARP1 rabbit IgG antibody provide flexible solutions for fixed-cell imaging and high-content analysis. The availability of both mouse and rabbit host species supports assay design flexibility and enables multiplexing with other primary antibodies, provided that species-matched, highly cross-adsorbed secondary antibodies are used.

Product information

Product name	PhenoVue anti-cleaved PARP1 - mouse IgG1 antibody	PhenoVue anti-cleaved PARP1 - rabbit IgG antibody
Part number	PAMSCPARP11	PARBCPARP11
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	IgG1, κ	IgG
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	PO4 pH7 100 mM - 0.1% BSA	PO4 pH7 100 mM - 0.1% BSA
Applications	High-content analysis (immunofluorescence, imaging, microscopy)	High-content analysis (immunofluorescence, imaging, microscopy)
Shipping conditions	Dry ice	Dry ice
Storage conditions	-16°C or below. Protect from light.	-16°C or below. Protect from light.

Stability

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

Protocols

Cell culture

Seeding cells: 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.

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

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 ready-to-use PhenoVue permeabilization solution (PVPERM051), 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 1h 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 anti-cleaved PARP1 mouse IgG1 antibody (1X) or anti-cleaved-PARP1 Rabbit IgG antibody (1X) diluted in PhenoVue Dye Diluent A (1X) and incubate for 3h at room temperature.
9. **Washing:** Wash three with PBS.
10. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluor Goat anti-Mouse Highly Cross-Adsorbed antibody or PhenoVue Fluor Goat anti-Rabbit Highly Cross-Adsorbed antibody, in accordance with the primary anti-cleaved PARP1 antibody species used, diluted in PhenoVue Dye Diluent A (1X) at 5-10µg/mL and incubate for 1h at room temperature, protected from light.

Optional: At this step, other reagents like PhenoVue Hoechst 33342 Nuclear Stain (1-2µg/mL) or PhenoVue Fluor 555 – Phalloidin (10-50nM) can be included in the same preparation mix.

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

TIPS

When multiplexing the PhenoVue cleaved PARP1 IgG antibodies with primary antibodies from other species, you can use the PhenoVue anti-Species Highly Cross-Adsorbed antibodies to help avoid any cross-reactivity between species.

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

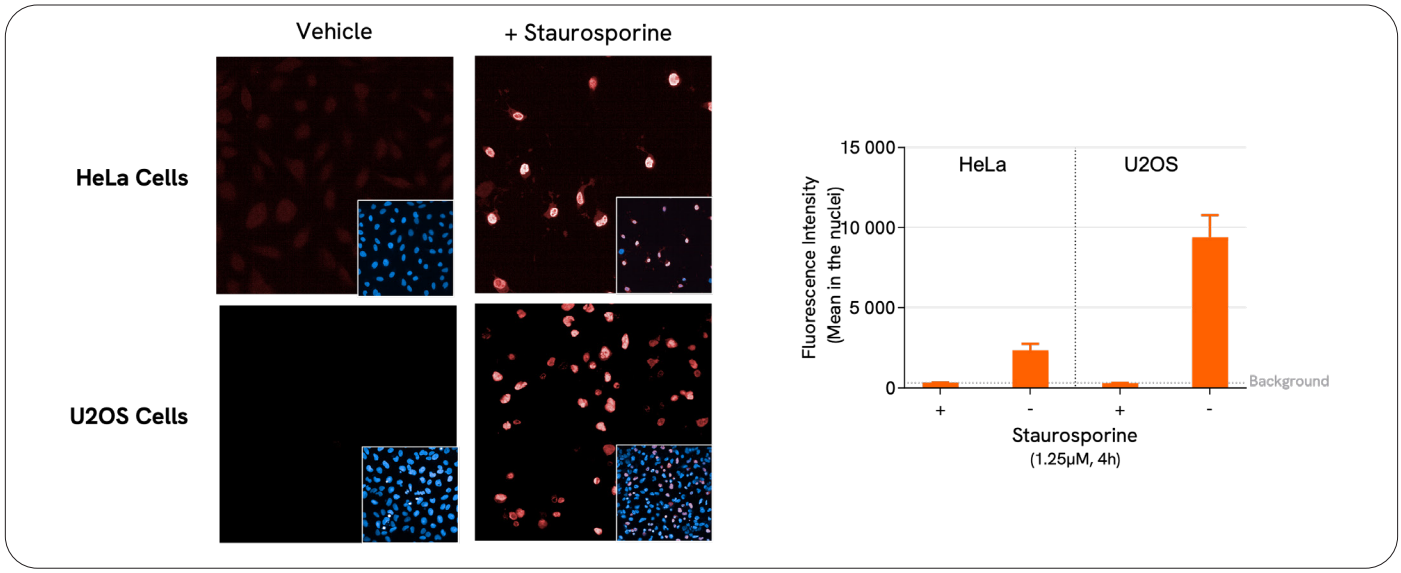


Figure 1: **PhenoVue anti-Cleaved PARP1 Rabbit IgG antibody specifically detects apoptosis induced by staurosporine treatment**

HeLa and U2OS cells were seeded in PDL-coated PhenoPlate™ 96-well microplates at a density of 10,000 cells per well and cultured overnight at 37 °C, 5% CO_2. Cells were then treated for 4 hours with either staurosporine (1.25 µM) or vehicle control (0.5% DMSO).

Following fixation, permeabilization, and blocking, cells were stained with the PhenoVue anti-Cleaved PARP1 Rabbit IgG antibody (1X, 3 h, RT) and detected using PhenoVue Fluor 647 Goat anti-Rabbit Highly Cross-Adsorbed secondary antibody (10 µg/mL, 1 h, RT) together with PhenoVue Hoechst 33342 Nuclear Stain (1 µg/mL). Images were acquired on the Operetta CLS™ high-content analysis system in confocal mode using a 40× water-immersion objective.

Representative images show low basal cleaved PARP1 signal in vehicle-treated cells and a strong nuclear apoptotic signal following staurosporine treatment in both HeLa and U2OS cell models. Quantification of mean nuclear fluorescence intensity confirmed robust induction of cleaved PARP1 upon apoptosis induction.

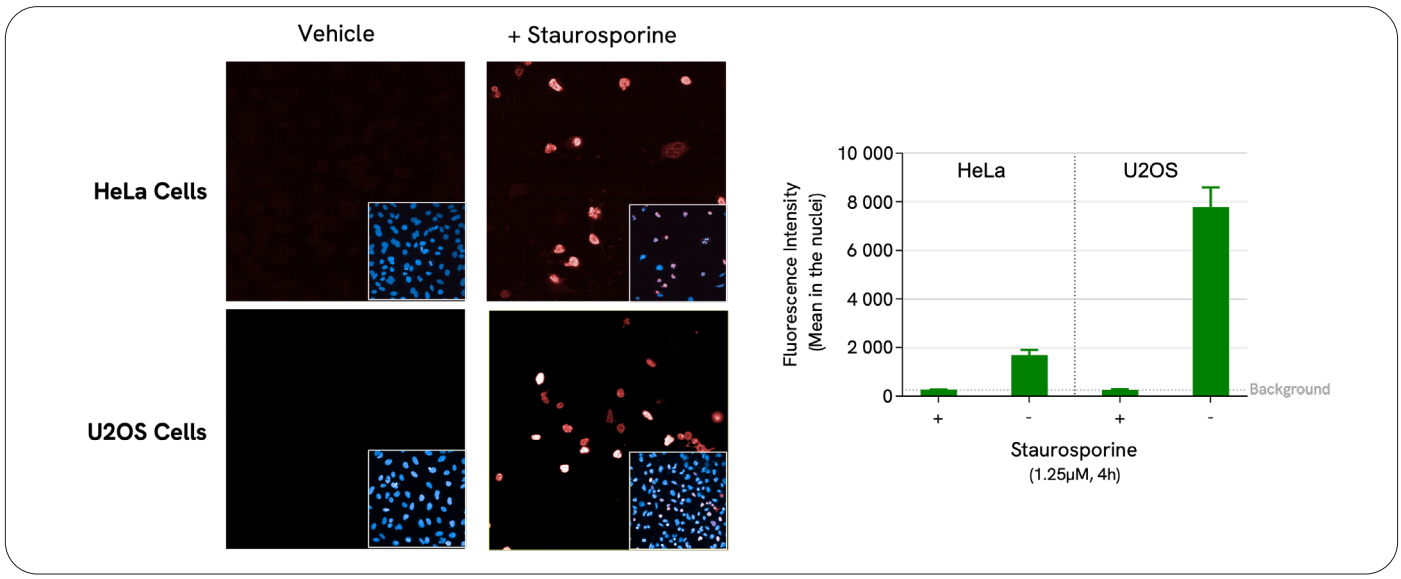


Figure 2: **PhenoVue anti-Cleaved PARP1 Mouse IgG1 antibody specifically detects apoptosis induced by staurosporine treatment**

HeLa and U2OS cells were seeded in PDL-coated PhenoPlate™ 96-well microplates at a density of 10,000 cells per well and cultured overnight at 37 °C, 5% CO_2. Cells were then treated for 4 hours with either staurosporine (1.25 µM) or vehicle control (0.5% DMSO).

Following fixation, permeabilization, and blocking, cells were stained with the PhenoVue anti-Cleaved PARP1 Mouse IgG1 antibody (1X, 3 h, RT) and detected using PhenoVue Fluor 647 Goat anti-Mouse Highly Cross-Adsorbed secondary antibody (10 µg/mL, 1 h, RT) together with PhenoVue Hoechst 33342 Nuclear Stain (1 µg/mL). Images were acquired on the Operetta CLS™ high-content analysis system in confocal mode using a 40× water-immersion objective.

Representative images show minimal basal cleaved PARP1 staining in vehicle-treated cells and a strong increase in nuclear apoptotic signal following staurosporine treatment in both HeLa and U2OS cell models. Quantification of mean nuclear fluorescence intensity confirmed robust induction of cleaved PARP1 upon apoptosis induction.

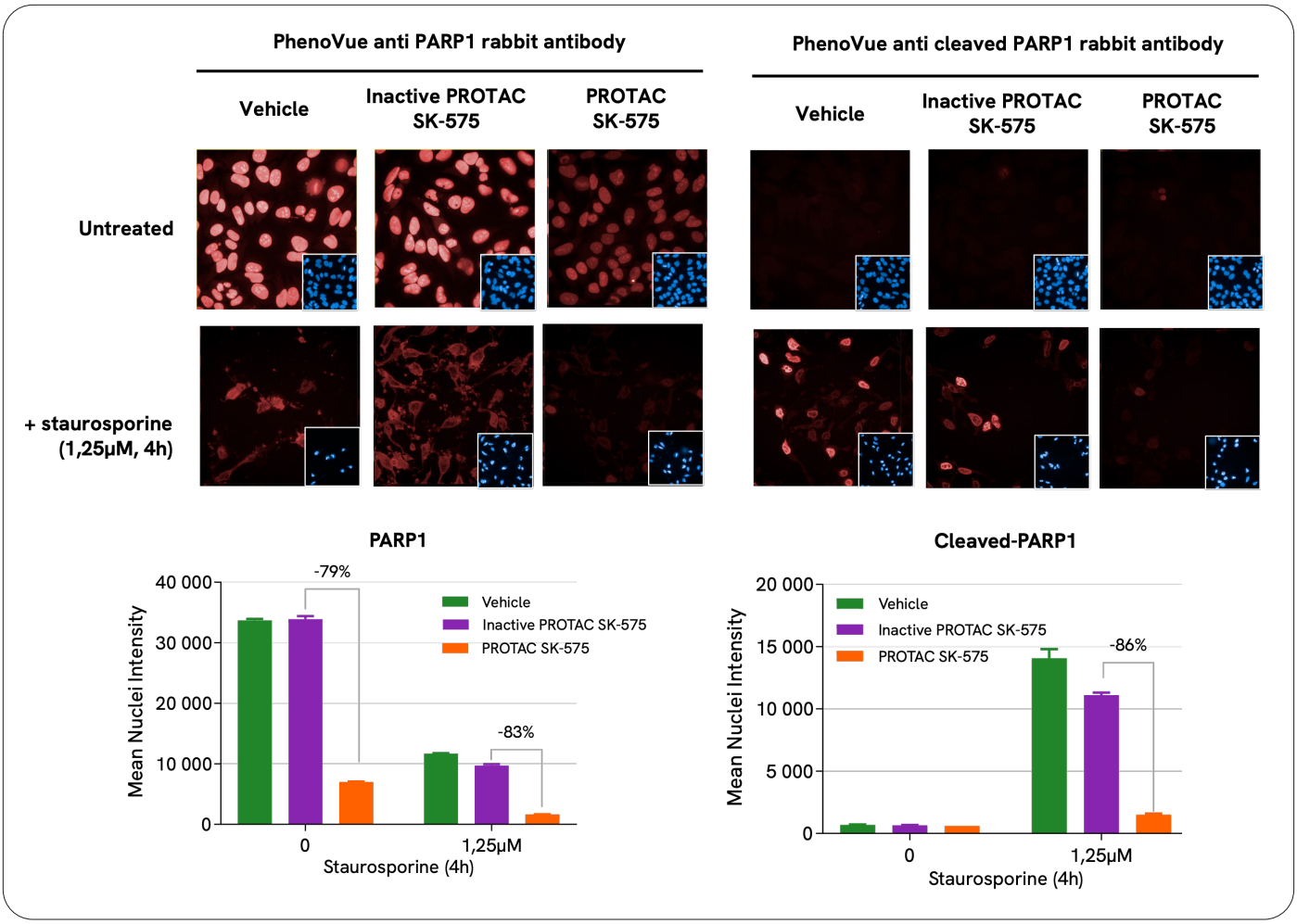


Figure 3: **PROTAC-mediated PARP1 degradation attenuates cleaved PARP1 detection during apoptosis**

HeLa cells were seeded in PhenoPlate™ 96-well microplates at a density of 10,000 cells per well and cultured overnight at 37 °C, 5% CO_2. Cells were then treated for 24 hours with the PARP1-targeting PROTAC SK-575 (100 nM) or with the inactive control PROTAC SK-575NEG (0.01% DMSO final concentration). During the last 4 hours of treatment, apoptosis was induced using staurosporine (1.25 µM).

Following fixation, permeabilization, and blocking, cells were stained either with the PhenoVue anti-PARP1 Rabbit antibody (left panel) or with the PhenoVue anti-Cleaved PARP1 Rabbit IgG antibody (right panel), both used at 1X for 3 h at room temperature. Detection was performed using PhenoVue Fluor 647 Goat anti-Rabbit Highly Cross-Adsorbed secondary antibody (1X, 1 h, RT) together with PhenoVue Hoechst 33342 Nuclear Stain (2 µg/mL).

Images were acquired on the Operetta CLS™ high-content analysis system in confocal mode using a 63× water-immersion objective for representative images and a 20× water-immersion objective for quantitative analysis.

Treatment with SK-575 induced a marked reduction in total PARP1 levels (–79% in untreated cells and –83% following staurosporine treatment), consistent with previously reported SK-575-mediated PARP1 degradation (Cao et al., J. Med. Chem., 2020). In the absence of staurosporine, cleaved PARP1 signal remained at background levels in both vehicle- and SK-575-treated cells, confirming that PARP1 degradation alone did not induce detectable apoptosis under these experimental conditions.

Following staurosporine treatment, a strong increase in cleaved PARP1 staining was observed in vehicle- and inactive PROTAC-treated cells, reflecting robust apoptosis induction. In contrast, SK-575-mediated degradation of PARP1 markedly reduced cleaved PARP1 accumulation (–86%), consistent with depletion of the PARP1 substrate available for apoptotic cleavage. These results demonstrate the specificity of the PhenoVue anti-Cleaved PARP1 antibody for the apoptotic cleaved form of PARP1.