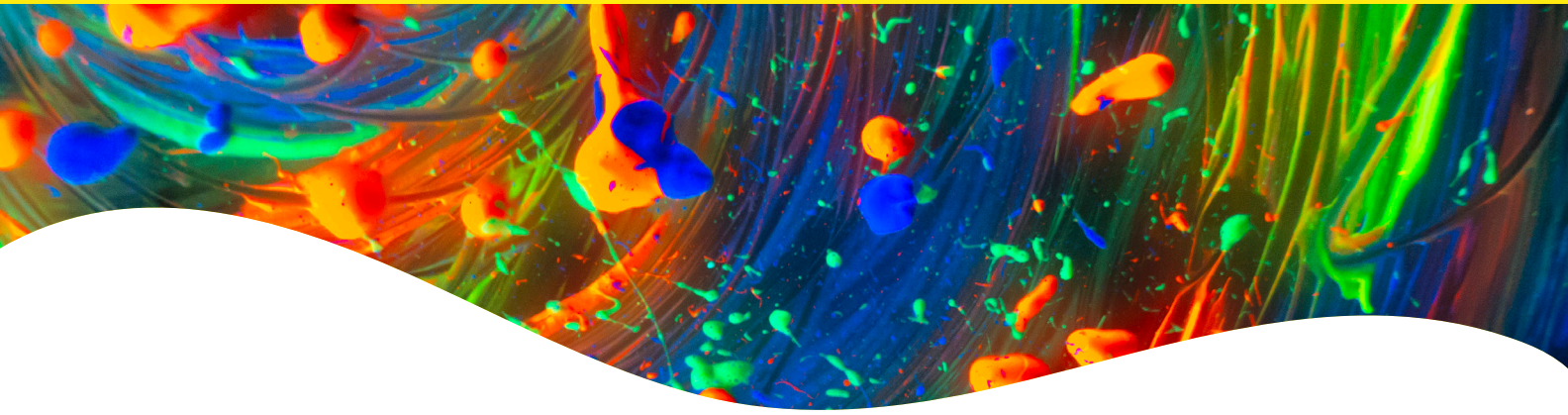
PhenoVue anti-53BP1 – rat IgG2a Antibody



Overview

DNA damage ranges from single-strand breaks (SSBs) to double-strand breaks (DSBs) and complex base modifications. DSBs are the most harmful, leading to genomic instability or cell death if unrepaired. Cells activate the DNA damage response (DDR) to detect and repair damage which is crucial for studying genome stability, assessing cytotoxic agents, and developing anticancer drugs. DSBs trigger the relocalization of key DDR proteins (MRE11/NBS1/RAD50, MDC1, 53BP1, BRCA1) to nuclear foci, where they interact with γH2AX. 53BP1 promotes canonical non-homologous end joining (C-NHEJ) over homologous recombination and alternative NHEJ.

The PhenoVue™ anti-53BP1 – rat IgG2a antibody, part of the PhenoVue DNA Damage Response Staining Kit (PDDR11), supports imaging and high-content analysis.

Product information

Product name	PhenoVue anti-53BP1 – rat IgG2a antibody
Part number	PART53BP111
Packaging	1 vial of 100 µL
Concentration	100x
Format	Liquid
Clonality	Monoclonal
Host species	Rat
Isotype	IgG2a, κ
Species	Human verified, mouse predicted
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Purification	Affinity chromatography
Formulation	PO_4 pH7 100 mM – 0.1% BSA
Applications	High content analysis (immunofluorescence, imaging, microscopy)
Shipping conditions	Dry ice
Storage conditions	-16°C or below. Protect from light.

Stability

- 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.
- 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 at 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 anti-53BP1 – rat IgG2a antibody and incubate for 3 h at room temperature.
9. **Washing:** Wash three with PBS.
10. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluor Goat anti-Rat antibody (5-10 µg/mL) and incubate for 1 h 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 (10-50 nM) 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.

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

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

Assay validation

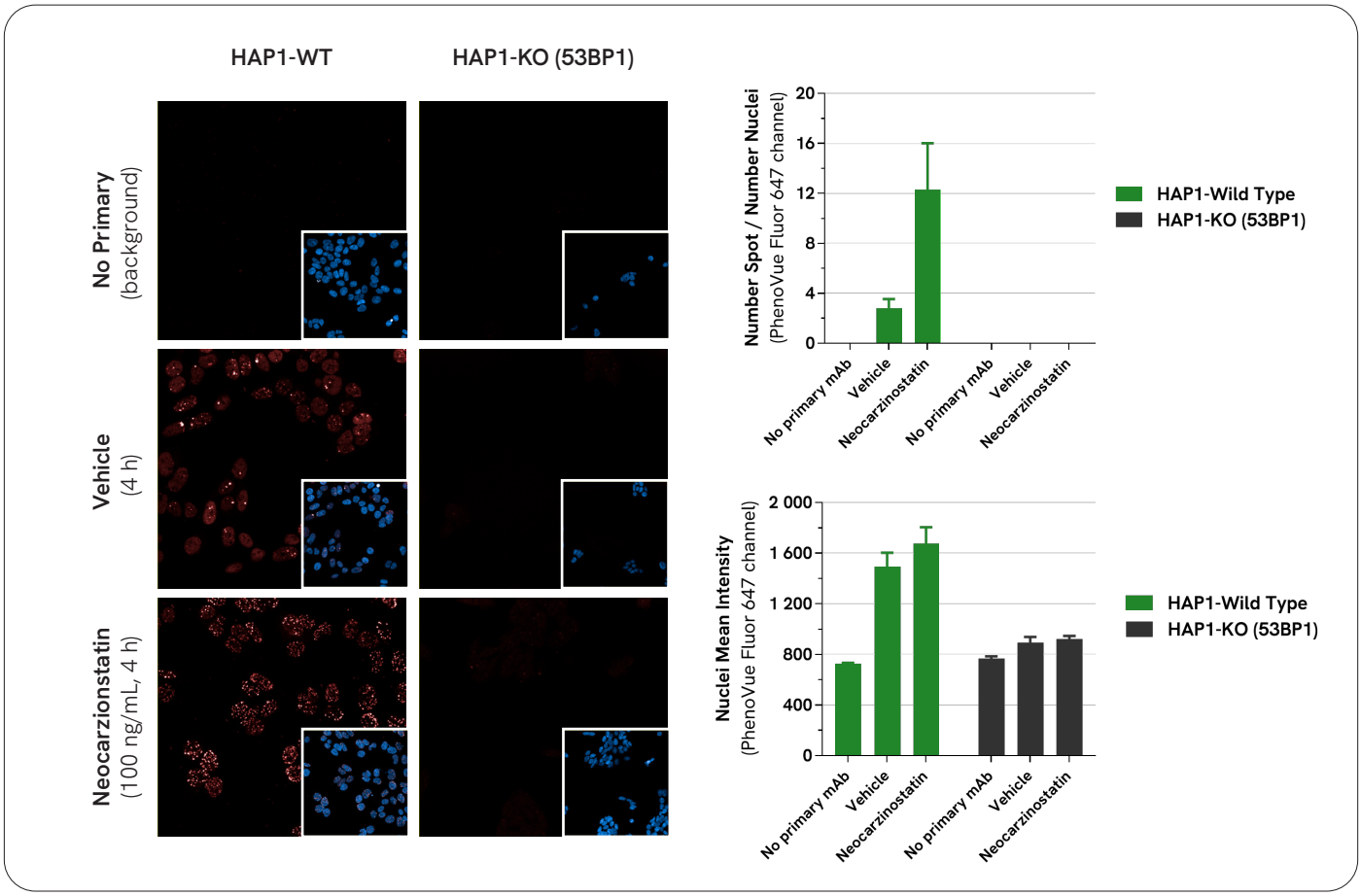


Figure 1: High Specificity of the Primary Anti-53BP1 Antibody

HAP1 wild-type (WT) and HAP1 53BP1-knockout (KO) cells (Revvity) were seeded in PhenoPlate 96-well microplates at a density of 20,000 cells per well and incubated overnight at 37 °C with 5 % CO_2 . Cells were then treated with either 100 ng/mL neocarzinostatin or vehicle control (0.02 % DMSO) for 4 hours at 37 °C with 5 % CO_2 . Following fixation, permeabilization, and blocking, cells were stained with the anti-53BP1 rat IgG2a primary antibody, followed by the PhenoVue Fluor 647 - goat anti-rat secondary antibody. Images were acquired using the Operetta CLS™ high-content imaging system with a 63× water immersion objective in confocal mode. The anti-53BP1 primary antibody demonstrated high specificity: No staining was detected in HAP1 53BP1-KO cells, confirming target specificity. Strong signal was observed in WT cells, validating its ability to detect endogenous 53BP1.

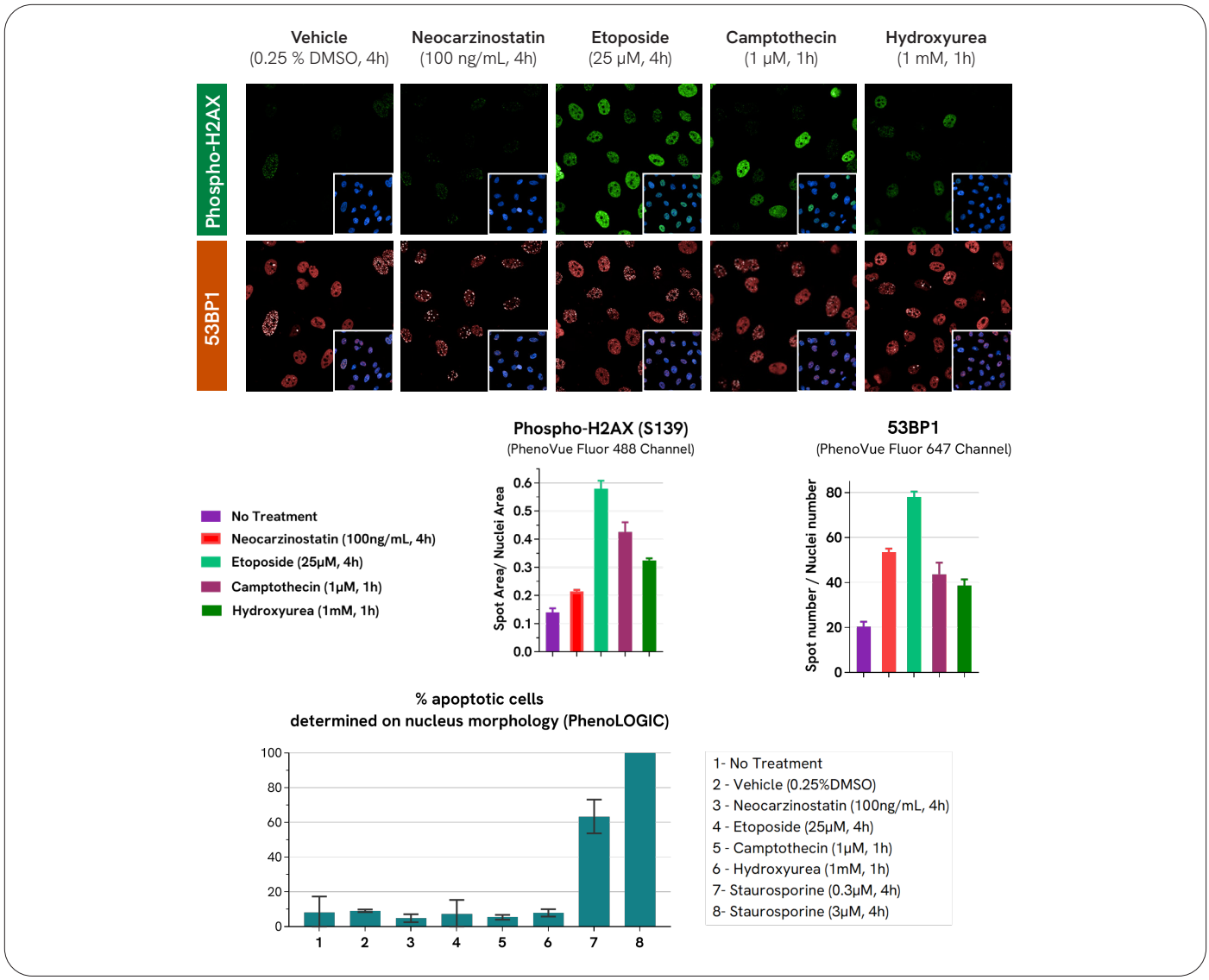


Figure 2: Deciphering DNA Damage Response Profiles

HeLa cells were 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 with various compounds at the indicated concentrations and incubation times. Following fixation, permeabilization, and blocking, the cells were stained using the PhenoVue DNA Damage Response Staining Kit. Images were acquired using the Opera Phenix™ Plus high-content imaging system with a 63× water immersion objective in confocal mode. Different compounds triggered distinct DNA damage response profiles:

- Phosphorylation of H2AX (S139) increased to varying degrees, leading to either discrete nuclear foci (e.g., neocarzinostatin), diffuse nuclear staining, or a combination of both (e.g., etoposide, camptothecin, hydroxyurea).

- 53BP1 relocalization was observed, shifting from a diffuse nucleoplasmic distribution to foci that largely colocalized with γH2AX at DNA damage sites.

- Nuclear morphology changes, assessed using the PhenoVue Hoechst 33342 channel, were analyzed with PhenoLOGIC™ (Harmony™ or Signals Image Artist™) to quantify late apoptotic cells (characterized by nuclear shrinkage and/or DNA fragmentation). PhenoLOGIC™ was trained using a positive control cell treated with 3 µM staurosporine for 4 hours.