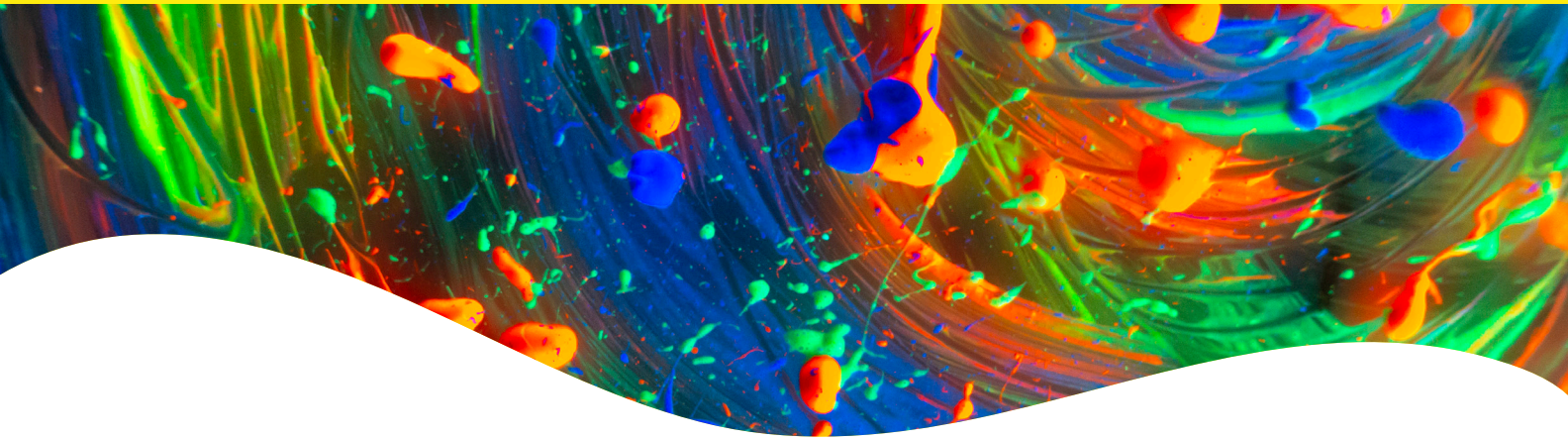
PhenoVue anti-p62/SQSTM1 – mouse IgG1 antibody



Overview

Autophagy is a fundamental catabolic process that enables cells to degrade and recycle damaged organelles, misfolded proteins, and other cytoplasmic constituents via lysosome-mediated degradation. It plays a key role in maintaining cellular homeostasis, adapting to metabolic stress, and regulating inflammation. Autophagy is tightly regulated by multiple signaling pathways and is dynamically modulated in response to environmental conditions such as nutrient deprivation, oxidative stress, or infection.

Dysregulation of autophagy has been implicated in a wide range of pathological conditions including cancer progression and resistance, neurodegenerative disorders (e.g., Alzheimer's, Parkinson's), infectious diseases, and inflammatory syndromes. Therefore, reliable tools to visualize and quantify autophagic activity are essential for both basic research and therapeutic development.

p62/SQSTM1 is a selective cargo adaptor that accumulates when autophagy is impaired, serving as a readout for autophagic degradation. The PhenoVue™ anti-p62/SQSTM1 – mouse IgG1 antibody is part of the PhenoVue Autophagy staining kit (part number: PAUT14) and can be used as an individual reagent in imaging and broader high-content analysis applications. It can be used in combination with the various colors of PhenoVue Fluor goat anti-mouse highly cross-adsorbed antibodies.

Product information

Product name	PhenoVue anti-p62/SQSTM1 – mouse IgG1 antibody
Part number	PAMSP621
Packaging	1 vial of 100 µL
Concentration	100x
Format	Liquid
Clonality	Monoclonal
Host species	Mouse
Isotype	IgG1, κ
Species	Human
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™ 96-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 50 µL per well of anti-p62/SQSTM1– mouse IgG1 antibody and incubate for 3 h at room temperature.
9. **Washing:** Wash three times with PBS.
10. **Fluorescent secondary antibody incubation:** Add 50 µL per well of PhenoVue Fluor Goat anti-mouse 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 (1-2 µg/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.

* PhenoPlate 384-well microplates may also be used. Adjust the cell density accordingly and reduce the corresponding volumes by 2-fold.

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

Assay validation

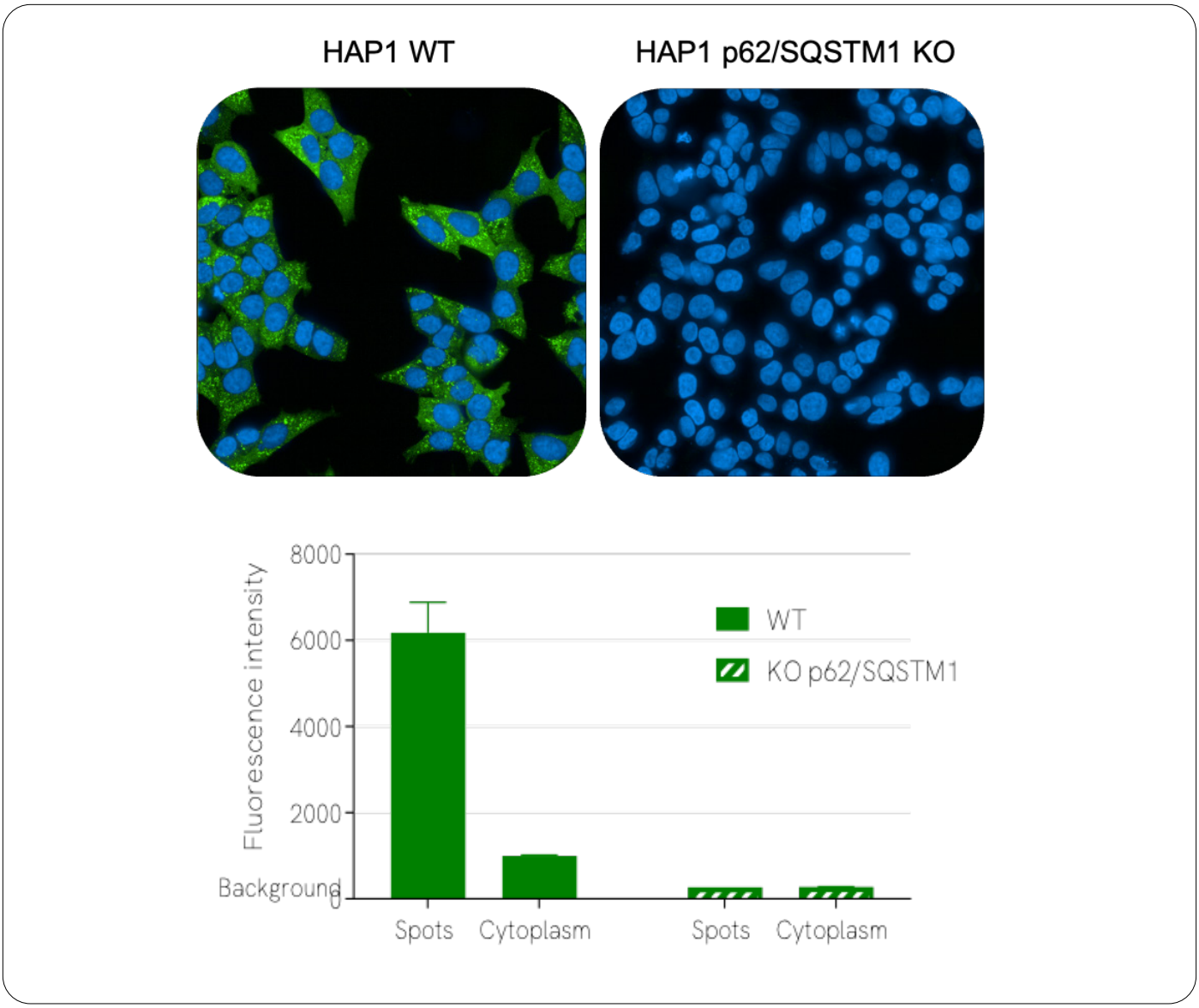


Figure 1. Specificity of the PhenoVue anti-p62/SQSTM1 antibody.

Figure 1 shows HAP1 wild-type (WT) and p62/SQSTM1 knockout (KO) cells seeded at a density of 15,000 cells per well and treated overnight with 25 µM Hydroxychloroquine. Following treatment, cells were stained using the PhenoVue anti-p62/SQSTM1 primary antibody, followed by the PhenoVue 488 Goat anti-mouse highly cross-adsorbed secondary antibody. Images were acquired using the Operetta CLS™ high-content analysis system (8-LED configuration) in confocal mode with a 63x water immersion objective.

As expected, no fluorescence signal was detected in the p62 KO cells, confirming the specificity of the PhenoVue anti-p62/SQSTM1 antibody for its target.

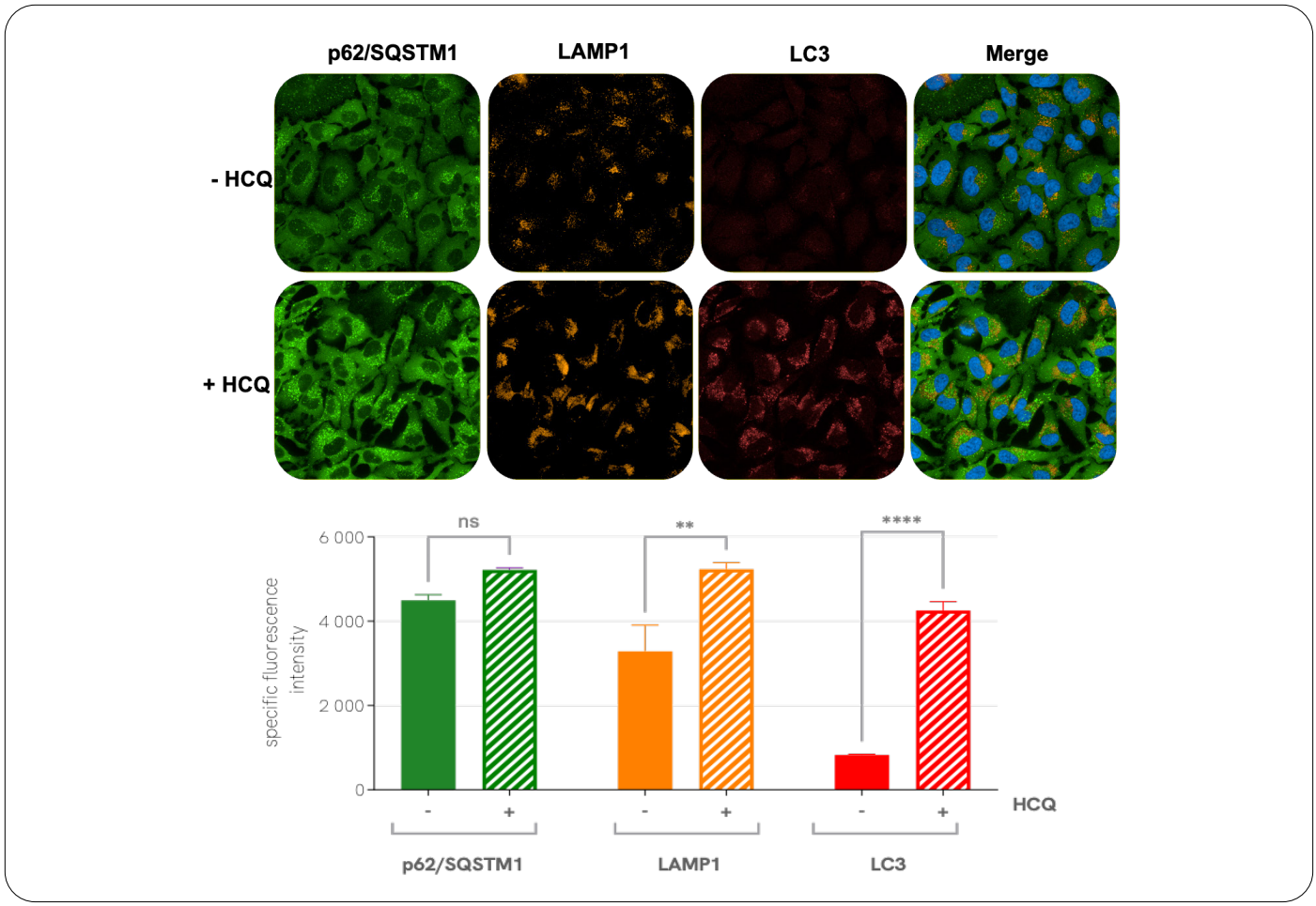


Figure 2. The PhenoVue autophagy staining kit monitors autophagy modulation at the single cell level.

Figure 2 shows HeLa cells seeded in PhenoPlate 96-well microplates at a density of 20,000 cells per well and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Following cell attachment, cells were treated or left untreated with 12.5 µM Hydroxychloroquine (HCQ) for 16 hours in complete growth medium. After treatment, cells were fixed, permeabilized, blocked, and stained using the PhenoVue Autophagy staining kit. Images were acquired using the Operetta CLS high-content analysis system (8-LED configuration), 63x water immersion objective.

In untreated cells, p62/SQSTM1, LAMP1, and LC3 signals appeared at baseline levels with limited puncta.

Following HCQ treatment, an increase in punctate staining was observed for LAMP1 and LC3, consistent with the accumulation of lysosomes and autophagosomes due to late-stage autophagy inhibition. Quantitative analysis confirmed a significant increase in LAMP1 intensity and a highly significant increase in LC3 intensity. In contrast, p62/SQSTM1 levels remained unchanged, suggesting that HCQ at this dose and timepoint did not lead to measurable accumulation of p62. These results confirm that HCQ disrupts autophagic degradation and validates the sensitivity of the PhenoVue Autophagy staining kit for monitoring autophagy modulation at single cell level.