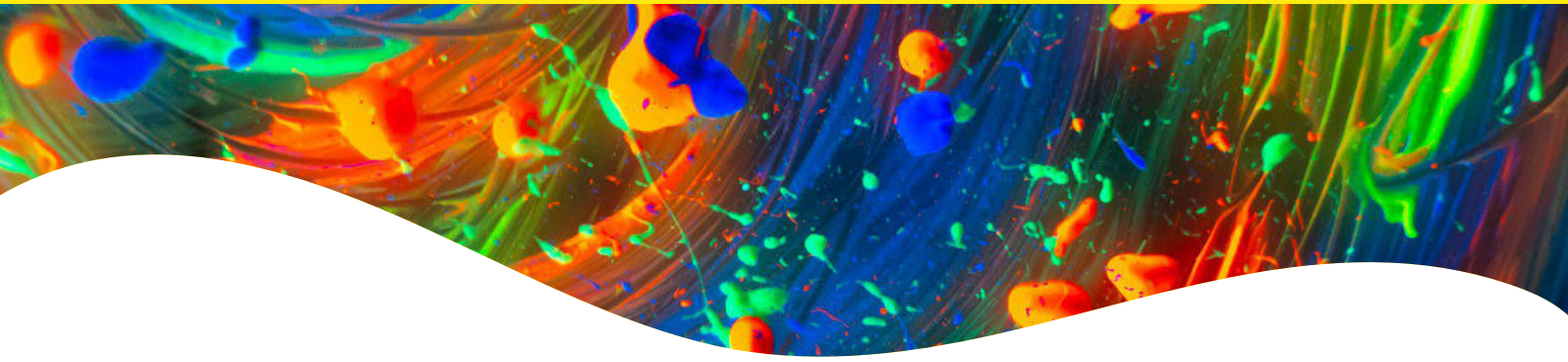
PhenoVue anti-LAMP1 antibody



Overview

LAMP1 (lysosomal associated membrane protein 1) is a major protein component of the lysosomal membrane and plays important roles in lysosome biogenesis and autophagy. LAMP1 is routinely used as a lysosome marker and LAMP1-positive organelles are often referred to as lysosomal compartments.

PhenoVue™ anti-LAMP1 antibody is part of the PhenoVue Multi Organelle Staining Kit and can be used as an individual reagent in imaging and broader high-content analysis applications.

Product information

Product name	PhenoVue anti-LAMP1 antibody
Part number	PALAMP11
Packaging	1 vial of 100 µL
Concentration	100X
Format	Liquid
Clonality	Monoclonal
Host species	Rat
Isotype	IgG2b
Cross-reactivity	Human only
Immunogen	Recombinant full length of Human LAMP1
Purification	Affinity chromatography
Formulation	PO_4 pH 7 100 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

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

1. **Fixation:** Add ready to use PhenoVue Paraformaldehyde 4% Methanol-Free Solution (PVPFA41) for 10-20 min at room temperature.
2. **Washing:** Wash three times with PBS for 5 min.
3. **Permeabilization:** Add PhenoVue Permeabilization solution diluted at 0.1% Triton X-100 in PBS, for 10 min at room temperature.
4. **Washing:** Wash three times with PBS for 5 min.
5. **Saturation:** Incubate with PBS-1% BSA for 1h at room temperature.
6. **Washing:** Wash three times with PBS for 5 min.
7. **Primary antibody incubation:** Add 25 µL per well of mouse monoclonal anti-LAMP1 antibody and incubate for 1-3h at room temperature or over night at 4°C.
8. **Washing:** Wash three with PBS for 5 min.
9. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluor goat anti-rat antibody and incubate for 1h at room temperature, protected from light.
10. **Washing:** Wash three times with PBS for 5 min.
11. Acquire images on an imaging device.

** PhenoPlate 96-well microplates may also be used. Adjust the cell density accordingly and increase 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

Validation data

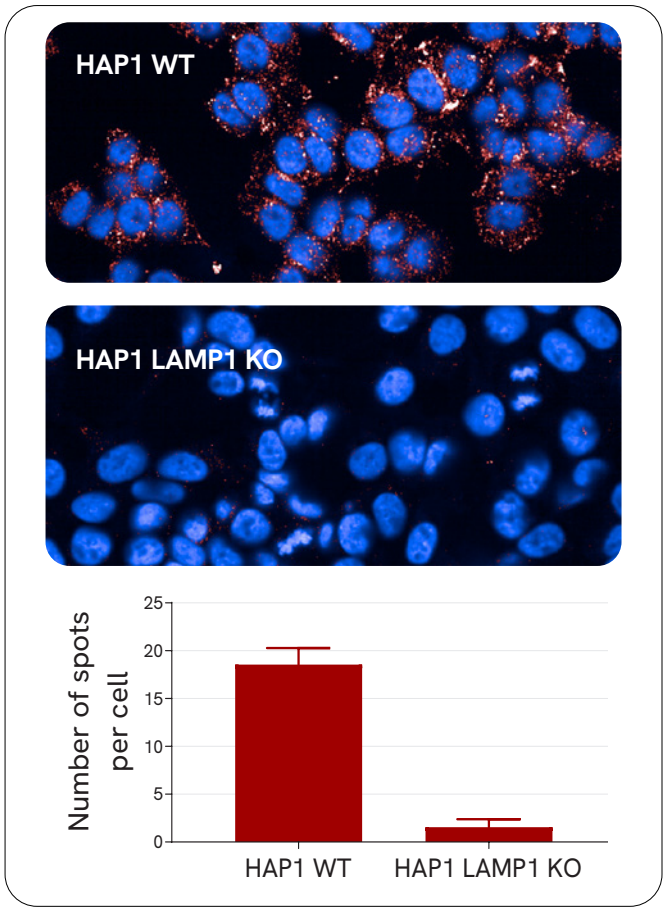


Figure 1: HAP1 WT and HAP1 LAMP1 KO were stained with PhenoVue anti- LAMP1 antibody and PhenoVue Fluor 647 Goat anti-Rat Highly Cross Adsorbed antibody. As expected, no fluorescent signal was detected in the LAMP1 Knock-Out cells, validating the specificity of PhenoVue anti- LAMP1 antibody.

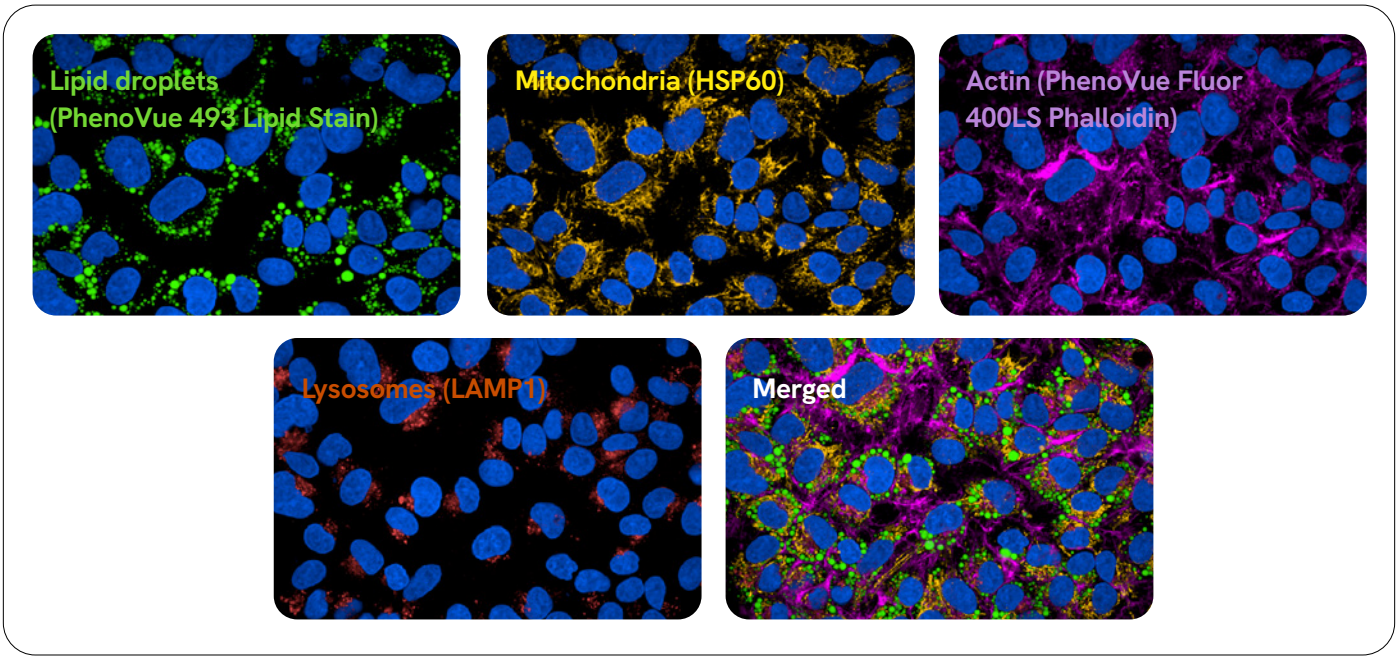


Figure 2: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were treated with oleic acid 200 µM for 24h to induce lipid droplets formation. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT), permeabilized (PhenoVue Permeabilization 0.1% Triton X-100 Solution – 10 min at RT) and stained using the PhenoVue Multi Organelle Staining Kit (part number: PMOS11). Images were acquired on the Opera Phenix® Plus (5 lasers) high-content analysis system with the 63X water objective.

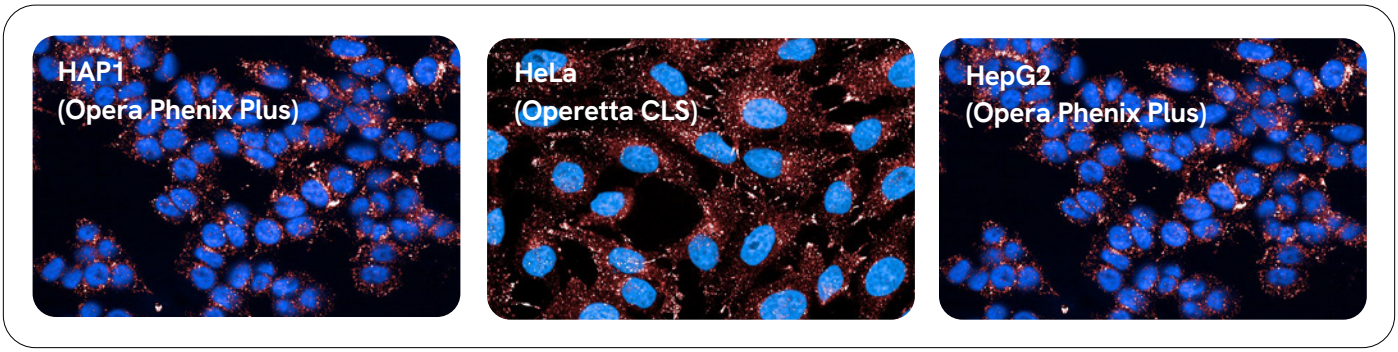


Figure 3: Various cellular models were stained with PhenoVue anti-LAMP1 associated with PhenoVue Fluor 647 goat anti-rat antibody Highly Cross-Adsorbed and PhenoVue Hoechst 33342. Images were acquired either on the Operetta® CLS™ or Opera Phenix Plus HCS instruments (63X objective).

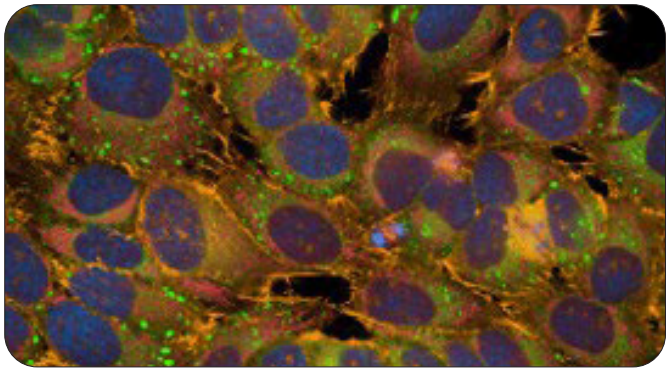


Figure 4: Phenotypic Cell Painting. HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 Mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated with a Cell Painting mix which includes PhenoVue 512 Nucleic Acid stain (3 µM), PhenoVue Hoechst 33342 nuclear stain (5 µg/mL), PhenoVue Fluor 568 - Phalloidin (33 nM), PhenoVue Fluor 488 - Concanavalin A (100 µg/ mL) and PhenoVue Fluor 555 - WGA for 30 min at RT. Images were acquired on the Operetta CLS high-content analysis system.

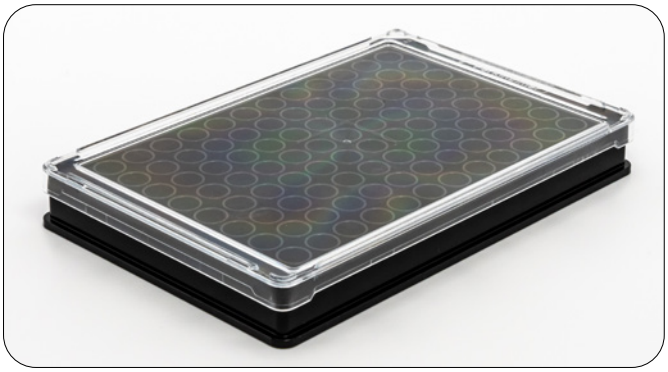


Figure 5: PhenoPlate 96-well microplate

Related products

PhenoVue Multi Organelle Staining Kit

PhenoVue anti-HSP60 antibody

PhenoPlate high-quality microplates for imaging

PhenoVue Cell Painting Kits

PhenoVue Fluor Secondary Antibody Conjugates

PhenoVue Organelle and Cell Compartment Stains

Opera Phenix Plus High-Content Screening System

Operetta CLS High-Content Analysis System

Harmony® Imaging and Analysis Software

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