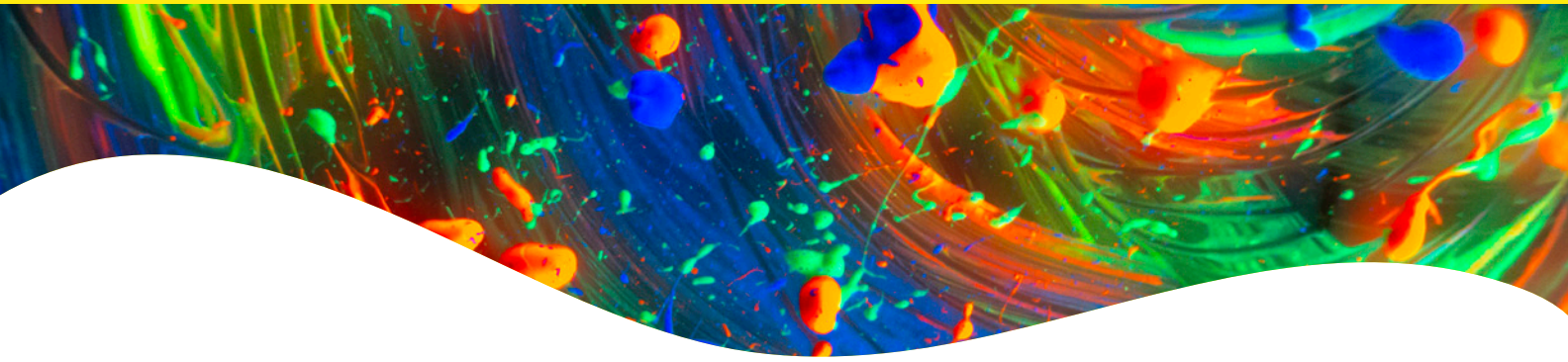
PhenoVue anti-HSP60 antibody



Overview

The mammalian molecular chaperone, HSP60 (Heat Shock Protein 60), is predominantly localized in mitochondria where it plays an essential role in protein homeostasis by assisting protein folding and assembly. Nevertheless, a small fraction of HSP60 resides in the cytosol as well as at the cell surface where its functions remain poorly understood.

PhenoVue™ anti-HSP60 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-HSP60 antibody
Part number	PAHSP601
Packaging	1 vial of 100 µL
Concentration	100X
Format	Liquid
Clonality	Monoclonal
Host species	Mouse
Isotype	IgG1
Cross-reactivity	Human, mouse, rat
Immunogen	Purified recombinant fragment of Human HSP60 in E. Coli
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 these products 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.

Protocol

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.
7. **Primary antibody incubation:** Add 25 µL per well of mouse monoclonal anti-HSP60 antibody and incubate overnight at 4 °C.
8. **Washing:** Wash three with PBS for 5 min.
9. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluors goat anti-mouse 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

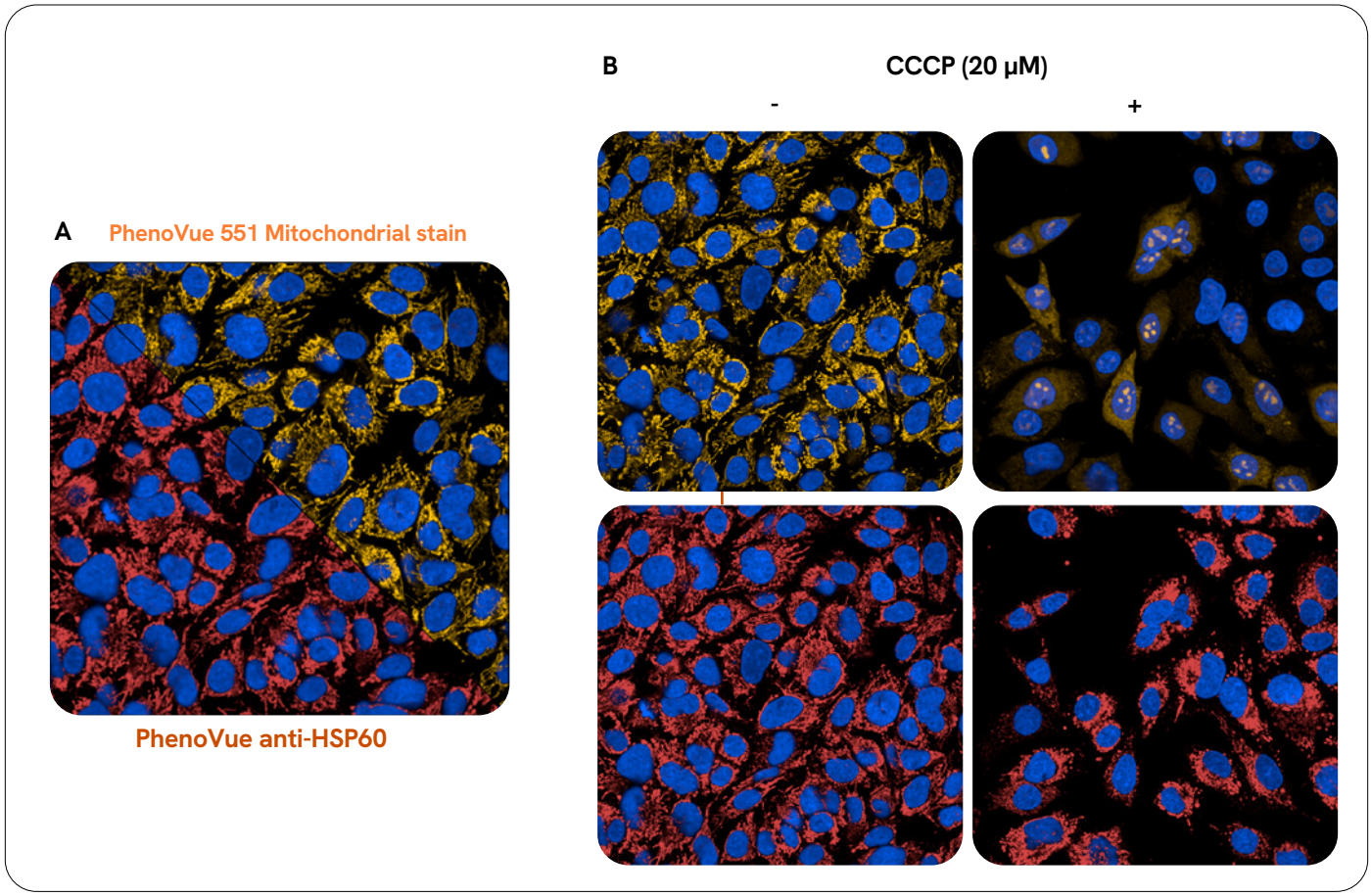


Figure 1: A - Typical tubular mitochondrial shapes with both PhenoVue anti- HSP60 antibody (Red) and PhenoVue 551 mitochondrial stain (Orange). B - To assess mitochondria specific staining of anti- HSP60 antibody, HepG2 cells were untreated and treated with CCCP compound which disrupts mitochondria membrane potential. As expected, CCCP treatment induces a decrease of the orange, fluorescent signal staining obtained with the PhenoVue 551 mitochondrial stain (sensitive to mitochondrial membrane depolarization), while the red fluorescent staining associated with anti-HSP60 antibody / PhenoVue Fluor 647 Goat anti-Mouse antibody is maintained.

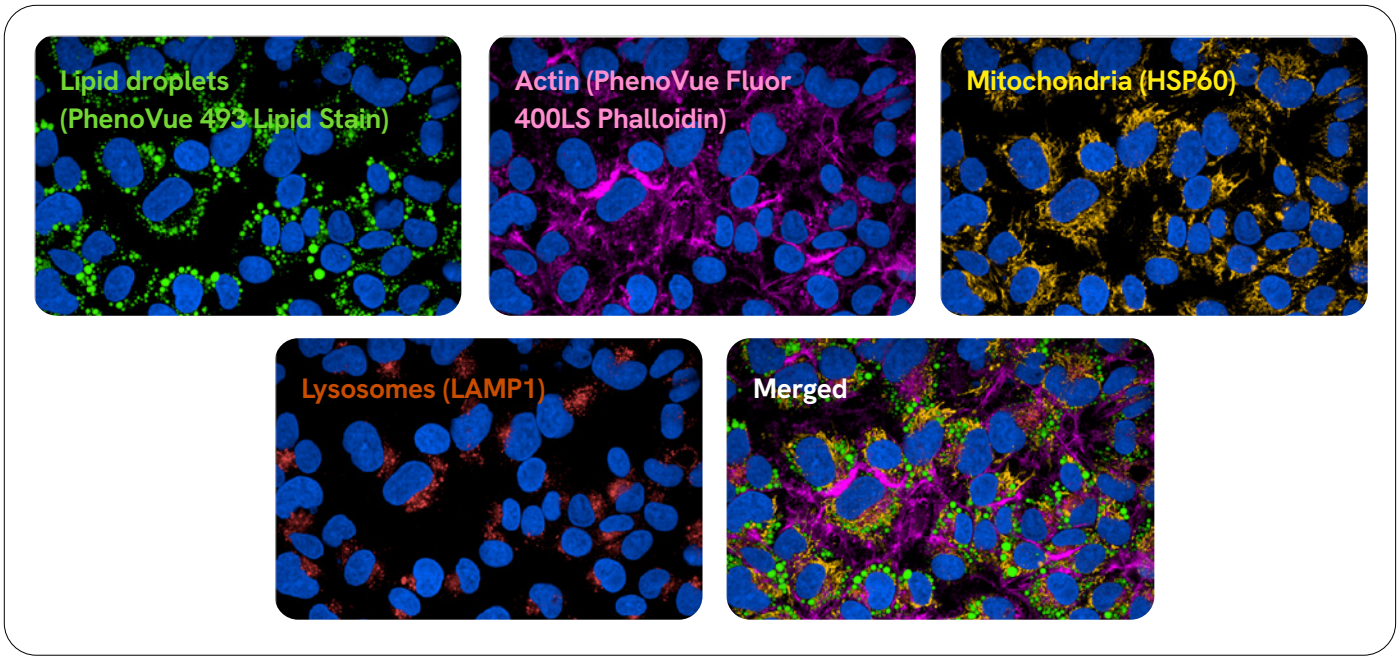


Figure 2: HepG2 cells were seeded in PhenoPlate 96-well microplates (20,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were treated with oleic acid 200 µM for 24h to induce lipid droplets formation. Cells were then fixed (PhenoVue Paraformaldehyde 4%, 20 min at RT), permeabilized (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.

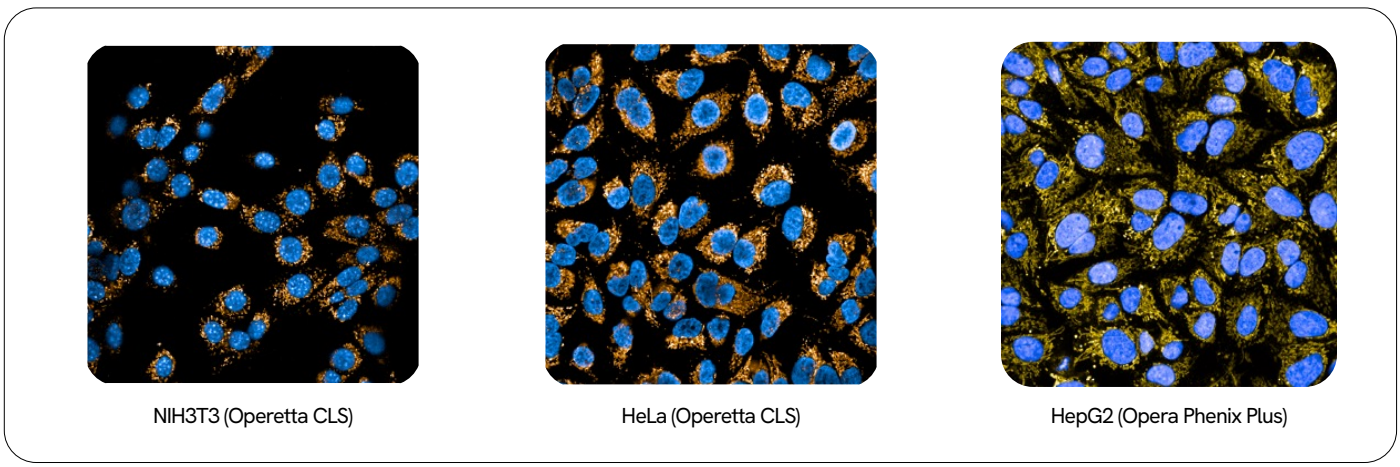


Figure 3: Various cellular models were stained with PhenoVue anti-HSP60 associated with PhenoVue Fluor 555 Goat anti-Mouse antibody Highly Cross-Adsorbed, and PhenoVue Hoechst 33342. Images were acquired either on the Operetta® CLS™ or Opera Phenix Plus HCS instruments (63X objective).

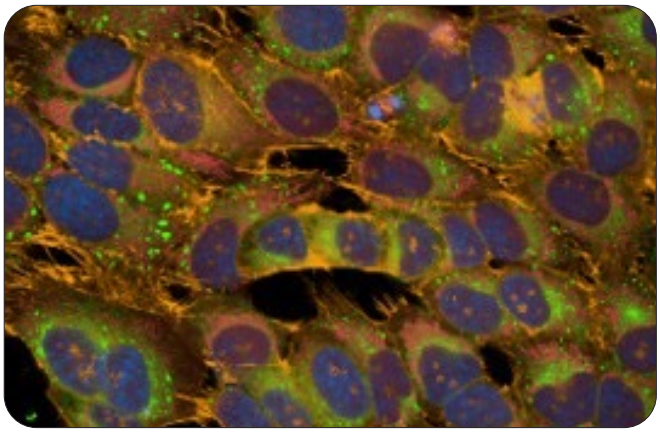


Figure 4: Phenotypic cell painting. HeLa cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 48h. Live cells were stained with PhenoVue 641 Mitochondrial stain (0.5 µM) for 30 min at 37 °C, then fixed and permeabilized. Next, cells were incubated 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.

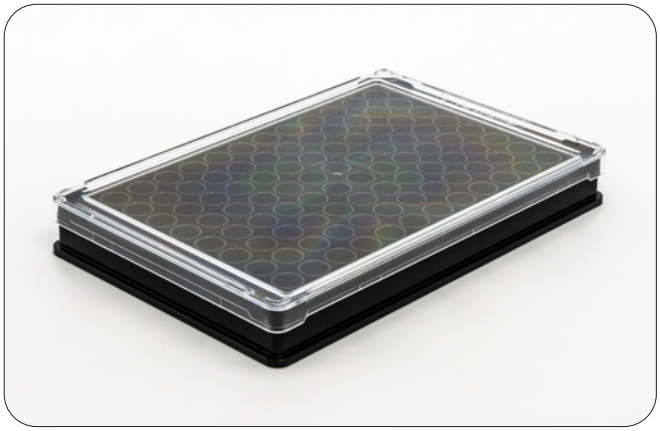


Figure 5: PhenoPlate 96-well microplate

Related products

PhenoVue Multi Organelle Staining Kit

PhenoVue anti-LAMP1 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

Revvity
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

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