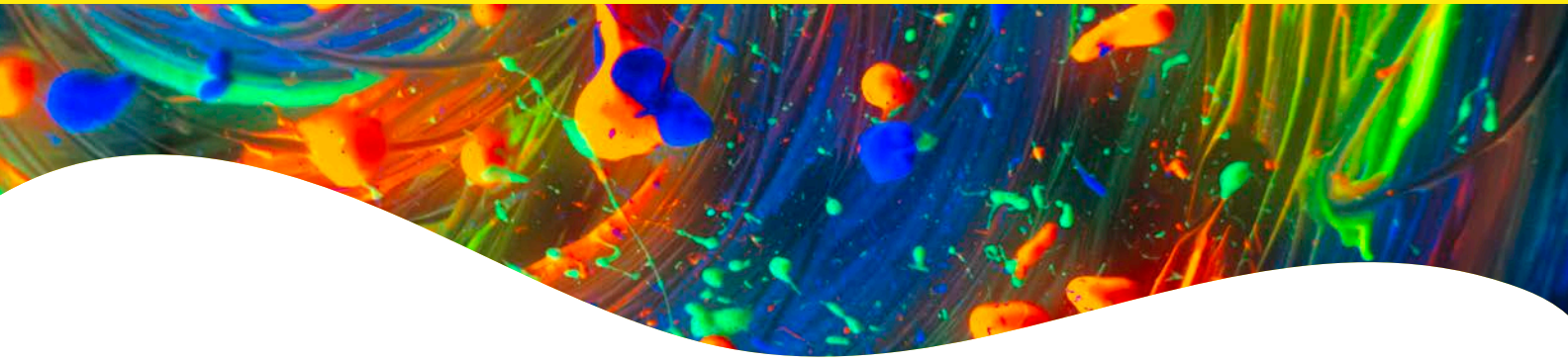
PhenoVue anti-TOM20 – mouse IgG1 antibody



Overview

Mitochondria are essential organelles responsible for cellular energy production, metabolism, and apoptosis regulation. Their morphology and integrity are tightly linked to cellular health and are dynamically regulated in response to metabolic stress, toxic insults, and signaling cues.

TOM20 is a key component of the mitochondrial protein import machinery. Localized on the outer mitochondrial membrane, TOM20 facilitates the recognition and translocation of proteins into mitochondria. Due to its outer membrane localization and expression, TOM20 is widely used as a reliable immunostaining marker for mitochondrial visualization.

The PhenoVue™ anti-TOM20 – mouse IgG1 antibody provides a convenient and robust solution for mitochondrial imaging and high-content analysis. It can be used as a standalone reagent or as an alternative to the anti-HSP60 antibody included in the PhenoVue Multi-Organelle Staining Kit (PMOS11). Substitution with anti-TOM20 significantly reduces primary antibody incubation time to 3 hours at room temperature while maintaining high staining performance.

Product information

Product name	PhenoVue anti-TOM20 – Mouse antibody
Part number	PAMSTOM2011
Packaging	1 vial of 100 µL
Concentration	100x
Format	Liquid
Clonality	Monoclonal (clone COD05B)
Host species	Mouse
Isotype	IgG1, κ
Species	Human verified, mouse and rat 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-TOM20 – mouse 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-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 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

Assay validation

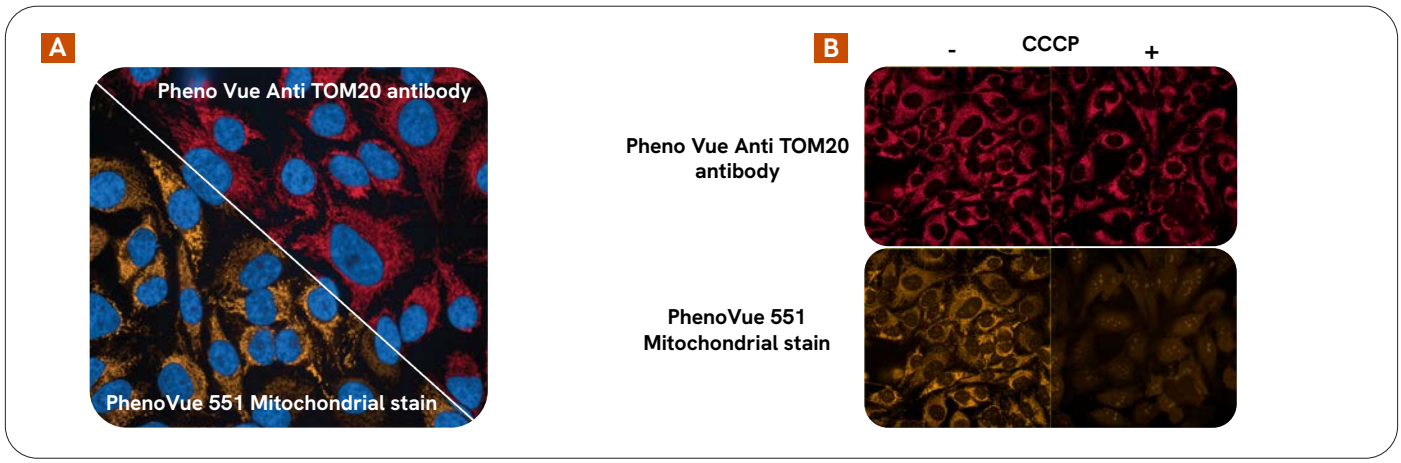


Figure 1: **PhenoVue anti-TOM20 antibody specifically labels mitochondria**
HeLa cells were seeded in 96-well PhenoPlates and treated with 20 µM CCCP for 1 h to disrupt mitochondrial membrane potential. Cells were stained with **PhenoVue™ 551 mitochondrial stain** during the last 30 min of treatment, fixed, and labeled with **PhenoVue™ anti-TOM20 antibody (1×)**. Images were acquired on the Operetta® CLS™ in confocal mode.
(A) PhenoVue anti-TOM20 (red) shows a typical tubular mitochondrial network and colocalizes with the PhenoVue 551 mitochondrial stain (orange).
(B) CCCP treatment induces a strong reduction of the PhenoVue 551 signal, while TOM20 staining remains clearly detectable, enabling membrane potential–independent mitochondrial visualization.

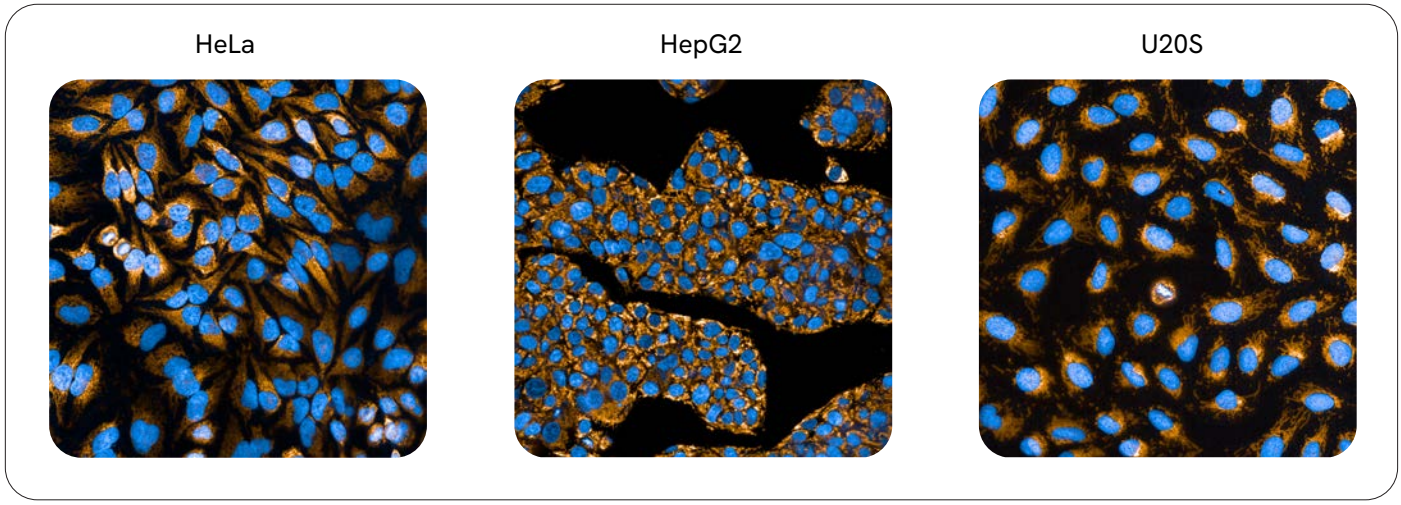


Figure 2: **TOM20 staining across multiple cell models**
HeLa, HepG2, and U2OS cells were seeded in 96-well PhenoPlates and cultured for 24 h at 37 °C. Following fixation and permeabilization, cells were stained with **PhenoVue anti-TOM20 antibody** (1×, 3 h, RT) and detected using **PhenoVue 555 goat anti-mouse secondary antibody.** Representative confocal images acquired on the Operetta CLS (40× objective) show robust and specific mitochondrial TOM20 staining across all three cell models.

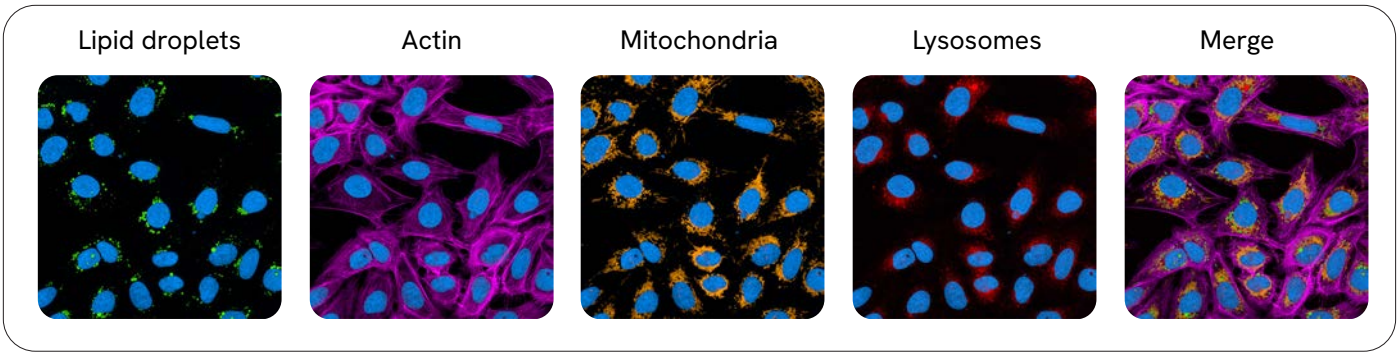


Figure 3: **Integration of TOM20 into the PhenoVue Multi-Organelle Staining Kit**

U2OS cells were seeded in 96-well PhenoPlates and cultured for 24 h at 37 °C. Following Oleic Acid treatment to induce lipid droplet formation, cells were fixed, permeabilized, and stained using the PhenoVue Multi-Organelle Staining Kit, with PhenoVue anti-TOM20 antibody (1×, 3 h, RT) replacing anti-HSP60.

Confocal images acquired on the Operetta® CLS™ (63× objective) show robust TOM20 mitochondrial staining together with LAMP1 (lysosomes), lipid droplets, actin, and nuclei, demonstrating seamless multiplexing and compatibility within the multi-organelle workflow.

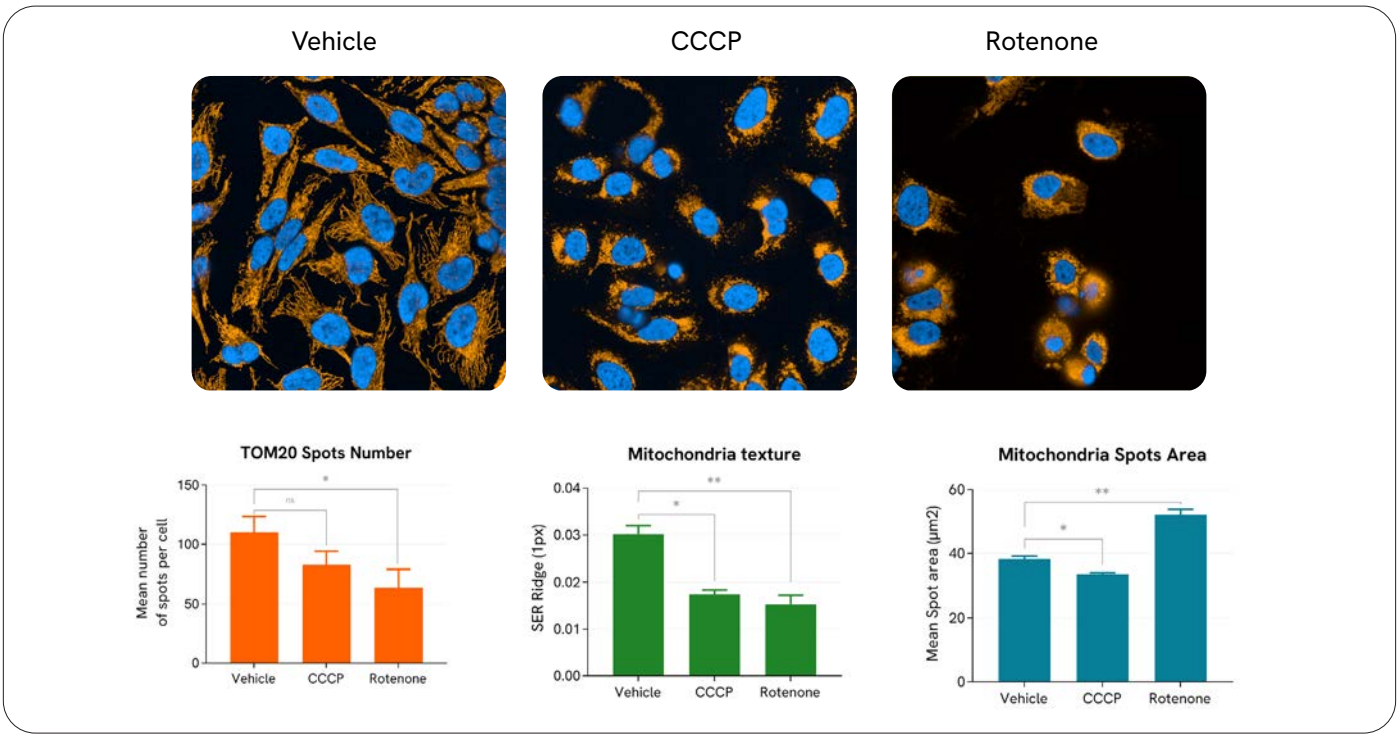


Figure 4: **Anti-TOM20 reveals drug-induced mitochondrial alterations**

HeLa and U2OS cells were seeded in 96-well PhenoPlates and treated with DMSO, 10 µM CCCP, or 10 µM rotenone. Following fixation and permeabilization, cells were stained with PhenoVue anti-TOM20 antibody (1×) and imaged on the Operetta CLS in confocal mode.

CCCP treatment induces mitochondrial depolarization and a pronounced shift from elongated networks to fragmented, punctate mitochondria, while rotenone triggers mitochondrial damage consistent with ROS induction. These distinct drug-specific effects are readily detected and quantified using TOM20-based mitochondrial imaging.