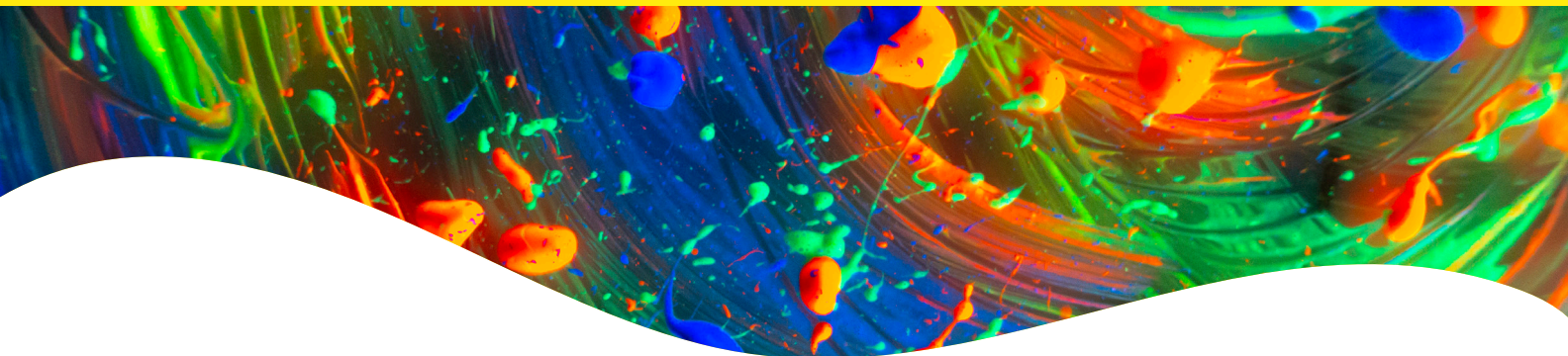


PhenoVue ROS, Total Oxidative Stress Indicators (ROS-490, ROS-570, and ROS-670)



Overview

Reactive oxygen species (ROS) are naturally produced by all living organisms and participate in cell signaling, gene expression and homeostasis. Increasing levels of ROS is associated with aging, as well as with various diseases such as diabetes, pulmonary disorders, inflammatory diseases, and cancer. ROS are also produced by exogenous sources such as UV, X-rays, smoking or infection. Ultimately elevated ROS concentration, which is known as oxidative stress, results in impairment of membrane lipids, proteins, and nucleic acids.

ROS are reactive molecules which contain oxygen and encompass various entities such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), hypochlorous acid (HOCl), peroxyxynitrite anion ($ONOO^-$), and nitric oxide (NO).

PhenoVue™ ROS total oxidative stress indicators, encompassing ROS-490, ROS-570 and ROS-670, enable a myriad of applications as well as multiplexing capability to unravel ROS related biological effects:

- PhenoVue ROS-490, total oxidative stress indicator is a non-fluorescent cell-permeant probe which emits bright green fluorescence upon reaction with hydroxyl radical, hydrogen peroxide and hypochlorite anion.
- PhenoVue ROS-570, total oxidative stress indicator is a non-fluorescent cell-permeant probe which emits bright orange fluorescence upon reaction with hydroxyl radical, hydrogen peroxide and superoxide anion.
- PhenoVue ROS-670, total oxidative stress indicator is a non-fluorescent cell-permeant probe which emits bright deep-red fluorescence upon reaction with hydroxyl radical, superoxide anion, hydrogen peroxide and hypochlorite anion.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue ROS-490, total oxidative stress indicator	CP15G	1	1 mg (2.362 μ mol)	Solid	Dry ice
PhenoVue ROS-570, total oxidative stress indicator	CP15O	1	1 mg (1.365 μ mol)	Solid	Dry ice
PhenoVue ROS-670, total oxidative stress indicator	CP15DR	1	1 mg (1.318 μ mol)	Solid	Dry ice

Storage and stability

- Store desiccated reagents at -16 °C or below, protected from light. Avoid repeated freeze / thaw cycles.
- 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.
- Allow the reagents to warm up to room temperature for 15 mins before opening the vials, and aliquot after reconstitution.
- Aliquoted reagents must be stored at -16 °C or below.

Equivalent number of microplates

Product name	When used at recommended concentration	96-well microplate (100 µL - 300 µL per well)	384-well microplate (25 µL - 90 µL per well)	1536-well microplate (4 µL - 12 µL per well)
PhenoVue ROS-490, total oxidative stress indicator	10 µM	Approx. 8 to 24	Approx. 8 to 24	Approx. 13 to 40
PhenoVue ROS-570, total oxidative stress indicator	10 µM	Approx. 4 to 14	Approx. 4 to 14	Approx. 7 to 22
PhenoVue ROS-670, total oxidative stress indicator	10 µM	Approx. 4 to 14	Approx. 4 to 14	Approx. 7 to 22

View our full range of high-quality imaging microplates at [Revvity.com](https://www.revivity.com)

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Reconstitution DMSO volume	Final concentration range*
PhenoVue ROS-490, total oxidative stress indicator	423.42 g/mol	10 mM	1 mg with 236.172 µL DMSO	1-10 µM
PhenoVue ROS-570, total oxidative stress indicator	732.81 g/mol	10 mM	1 mg with 136.461 µL DMSO	1-10 µM
PhenoVue ROS-670, total oxidative stress indicator	758.85 g/mol	10 mM	1 mg with 131.778 µL DMSO	1-10 µM

*Dilutions can be done in Hepes based buffer such as HBSS or PhenoVue dye diluent A.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Filter set	Quantum yield
PhenoVue ROS-490, total oxidative stress indicator	498	517	FITC	85%
PhenoVue ROS-570, total oxidative stress indicator	574	588	Cy3/TRITC	15%
PhenoVue ROS-670, total oxidative stress indicator	651	670	Cy5	nd*

*not determined

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/Permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue ROS-490, total oxidative stress indicator	Yes	No	No
PhenoVue ROS-570, total oxidative stress indicator	Yes	No	No
PhenoVue ROS-670, total oxidative stress indicator	Yes	No	No

Protocols

Cell culture

Seed cells in imaging black wall, clear bottom microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO₂ until 50-70% confluency.

Treat the cells with desired ROS inducing compounds (see Tips section).

Staining

The following protocol is provided as general guidelines. Optimal final concentration and staining duration may vary depending on the cell types and must be determined accordingly.

1. Add 1 to 10 µM PhenoVue ROS-490, ROS-570 or ROS-670 total oxidative stress indicator final concentration to your cells.
2. Incubate at 37 °C, 5% CO₂ for 15 to 60 minutes.
3. Replace the staining solution with fresh HBSS or any other appropriate medium.
4. Measure fluorescence using an imaging system such as the Opera Phenix® Plus high-content screening system.

Tips

- BSA and phenol red can affect PhenoVue ROS-490, total oxidative stress indicator fluorescence and should be used with caution.

Reactive Oxygen Species (ROS)	Compounds inducing ROS production
H ₂ O ₂	100 µM of H ₂ O ₂
•O ₂ ⁻	100 µM of KO ₂
¹ O ₂	100 µM of 3-(1,4-dihydro-1,4-epidioxy-1-naphthyl) propionic acid
OCl ⁻	3 µM of OCl ⁻
•OH	100 µM of ferrous perchlorate (II) and 1 mM of H ₂ O ₂
ONOO ⁻	3 µM of ONOO ⁻
NO	100 µM of 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene
ROO•	100 µM of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)
Auto-oxidation	2.5 hours of exposure to a fluorescent light source

Safety information

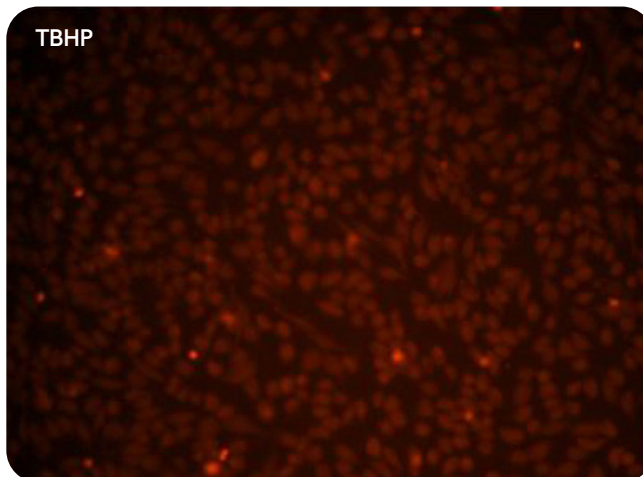
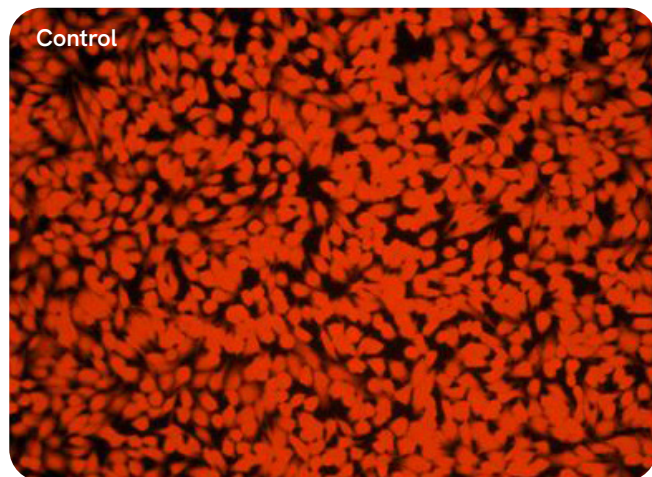
Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Applications

- High-content analysis/high-content screening
- Imaging microscopy

Validation data

PhenoVue ROS-570, total oxidative stress indicator



PhenoVue ROS-670, total oxidative stress indicator

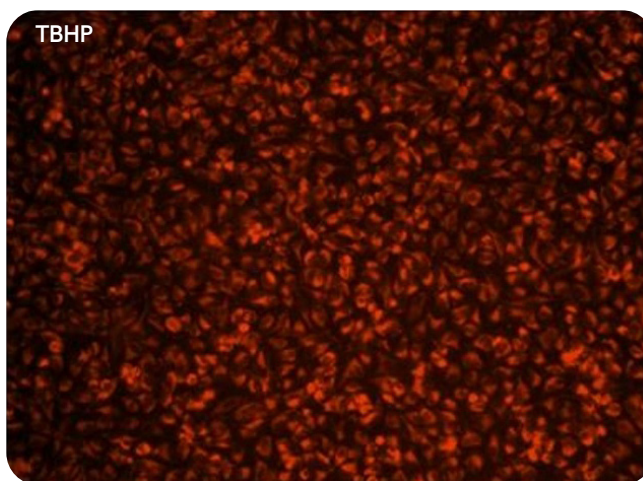
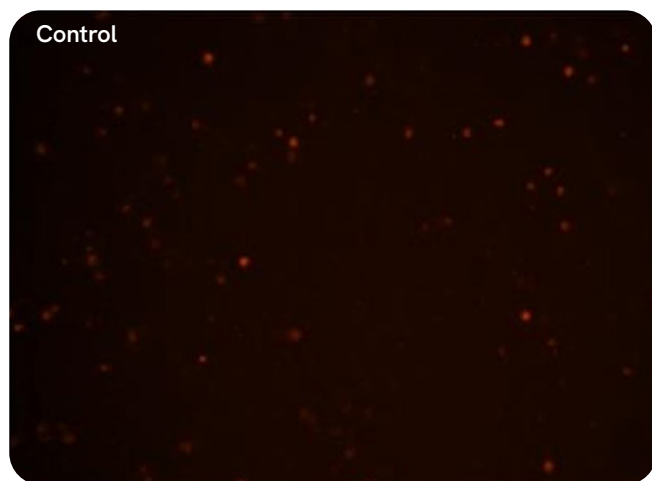
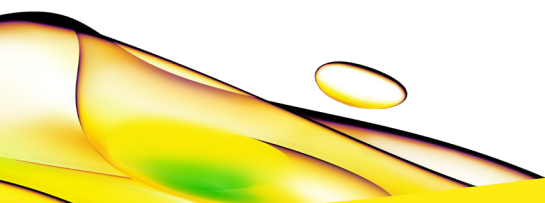


Figure 1: HeLa cells were seeded in 96-well imaging microplates and treated with 100 μ M tert-butyl hydroperoxide (TBHP) for 30 minutes before staining with **PhenoVue ROS-570 or ROS-670, total oxidative stress indicator**.



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