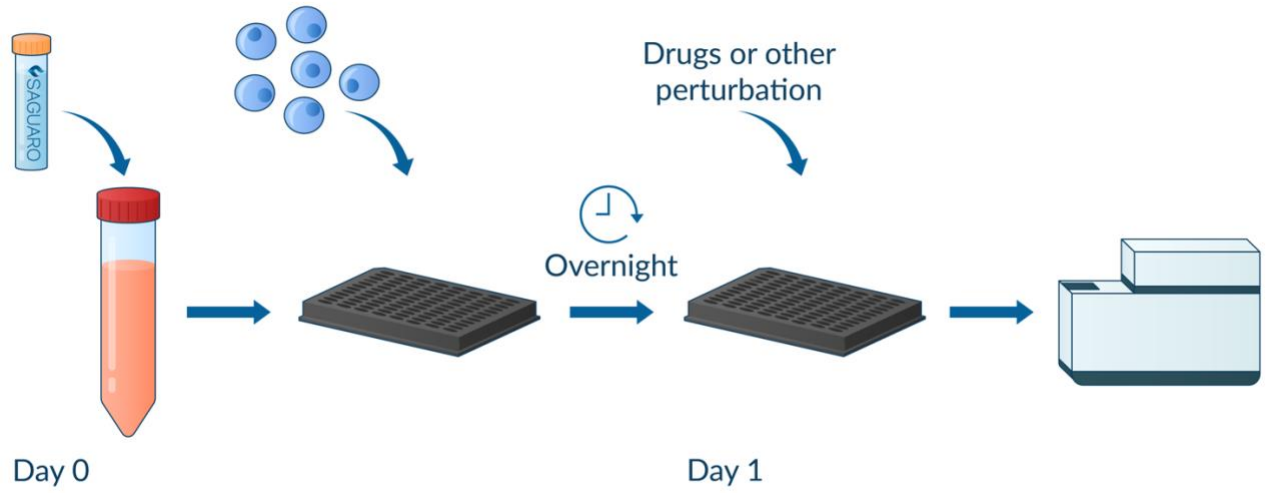
SAGUARO

ChromaLive™ Dye
New insight into cell physiology

Product Information Sheet

1. Protocol Overview



2. Content and Storage

Product	Content	Storage	Stability
ChromaLive™ Non-Toxic Dye	Diluted in 10uL of DMSO	• 4°C • Delivered at room temperature • Protect from light	1 year

Table 1. ChromaLive™ Product Information

Intended Use

For research use only. Not for use in diagnostics or therapeutic procedures.

3. General Guidelines

ChromaLive dye dilution and preparation

- Warm up the ChromaLive dye tube to room temperature before use to avoid condensation to form and water to get into the anhydrous dye solution
- Gently spin the tube before use to collect any dye solution that may remain near the cap
- Dilute ChromaLive dye in preferred culture medium (1:1000 the provided solution). Vortex thoroughly.

Cell culture protocol and compound testing, with ChromaLive dye

- Seed cells at desired density (typically to achieve 70-80% confluence) on imaging support, in previously prepared culture medium with ChromaLive dye
- Add control compounds, test compounds and negative controls for phenotypes of interest.
- NOTE: ChromaLive dye can also be added after cell seeding and compound addition. In that case, we recommend running a preliminary imaging test to validate the staining kinetics for your cells. As reference, ChromaLive staining stabilizes after 12 hours in U2OS cells.

Imaging

- NOTE: Keep ChromaLive dye in solution while imaging, no need for a washing step.
- NOTE: Nuclear staining can be added for cell segmentation during image analysis. Check manufacturer's guidelines.
- IMPORTANT IMAGING PARAMETERS
    - 2 wavelengths (Recommended)
      ChromaLive dye needs to be imaged at 2 different wavelengths minimally: ChromaLive561, *and either* ChromaLive488_Yellow *or* ChromaLive488_Red. That is because ChromaLive488_Yellow and ChromaLive488_Red look mostly similar (see figure 2), but can still provide slightly different information. However, selecting only one of these two is sufficient for differentiating between cellular phenotypes, even when phenotypes only have subtle differences.
    - 3 wavelenghts (Optional)
      ChromaLive dye can be imaged at 3 different wavelengths: If possible, both ChromaLive488_Yellow and ChromaLive488_Red can be used.
- ChromaLive wavelengths
    - ChromaLive488_Yellow: excitation at 488nm, image acquisition between 550-630nm
    - ChromaLive488_Red: excitation at 488nm, image acquisition between 630-750nm, and;
    - ChromaLive561: excitation at 561nm, image acquisition between 590-630nm

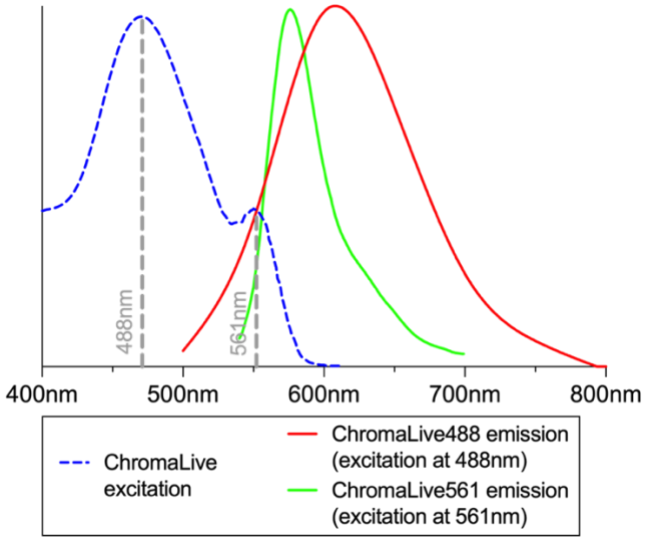


Figure 1. *Excitation and emission spectra of ChromaLive. Of note: ChromaLive is excited at 488nm and 561nm, with different resulting emission spectra. In green, ChromaLive561: emission spectrum when excited around 561nm, in red, ChromaLive488: emission spectrum when excited around 488nm).*

4. Image Examples

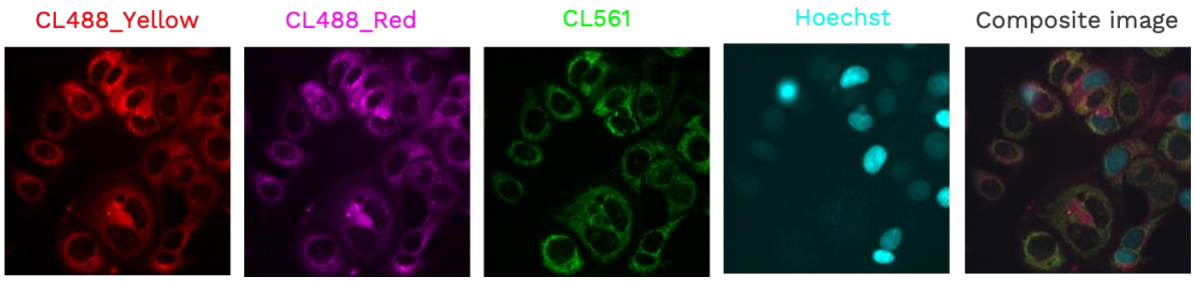


Figure 2. *MCF-7 cells stained with ChromaLive when seeded (24h before treatment). Images were acquired on Opera QEHS (scale bar not known). Red: ChromaLive 488-Yellow, Magenta: ChromaLive 488-Red, Green: ChromaLive 561-Yellow, Blue: Hoechst 33342.*

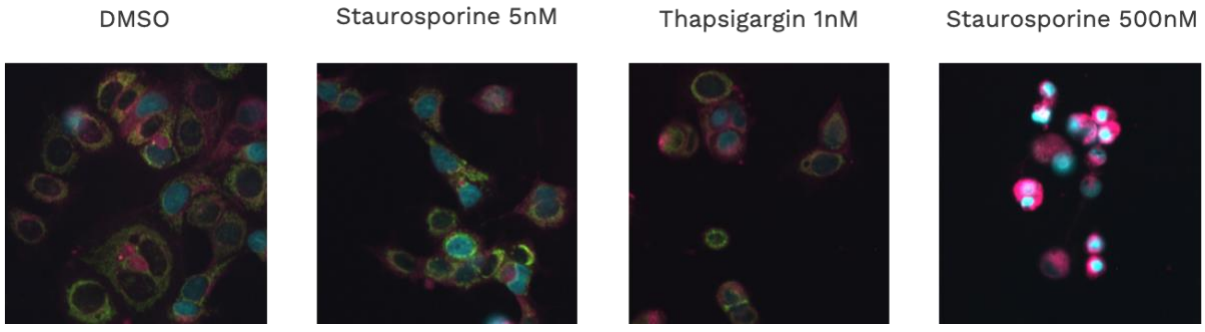


Figure 3. *MCF-7 cells with ChromaLive at 24h in different conditions. Images are composite images of cells treated for an early apoptotic phenotype (staurosporine 5nM), a stress phenotype (Thapsigargin 1nM), and an apoptotic/necrotic phenotype (staurosporine at 500nM), in comparison with an untreated DMSO control. Red: ChromaLive 488-Yellow, Magenta: ChromaLive 488-Red, Green: ChromaLive 561-Yellow, Blue: Hoechst 33342.*

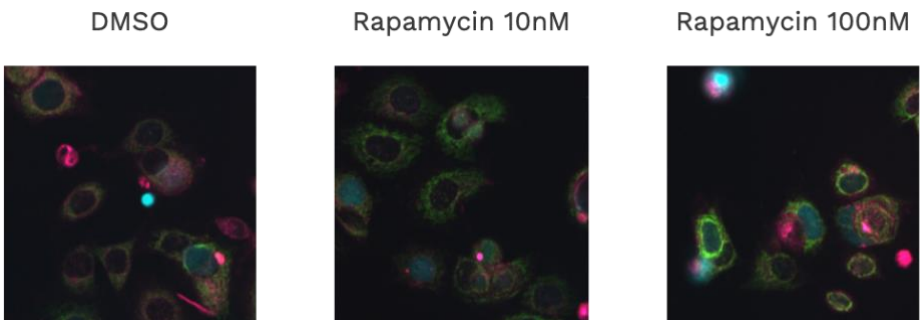


Figure 4. *MCF-7 cells with ChromaLive at 72h treated with Rapamycin. Images are composite images of cells treated for an autophagy phenotype at 10nM and 100nM of rapamycin, in comparison with an untreated DMSO control. Red: ChromaLive 488-Yellow, Magenta: ChromaLive 488-Red, Green: ChromaLive 561-Yellow, Blue: Hoechst 33342.*

5. Standard Protocol (for kinetic, 2D, live-cell assay)

MCF-7 cells, with standard compounds for apoptosis, ER stress and autophagy

ChromaLive dye dilution and preparation (day0):

- Warm up the ChromaLive dye tube to room temperature before use to avoid condensation to form and water to get into the anhydrous dye solution
- Gently spin the tube before use to collect any dye solution that may remain near the cap
- Dilute 10µL ChromaLive dye in 10mL culture medium (1:1000 the provided solution). Vortex thoroughly.
- NOTE: Culture medium here is RPMI 1640 complemented with 10% FBS and 1% Penicillin/Streptomycin

Cell culture protocol with ChromaLive dye (day0):

- Harvest and count MCF-7 cells
- Resuspend cells in prepared culture medium with ChromaLive dye at 80,000 cells/mL
- Seed 96 well plate with 100µL cell suspension per well, for a final amount of 8,000 cells per well
- Incubate overnight at 37°C, 5% CO2.

(OPTIONAL) Hoechst labeling of nucleus, for cell segmentation (day1)

- Dilute Hoechst 33342 solution at 1µg/mL in culture medium, add 12.5µL per well for a final concentration of 100ng/mL. Incubate for at least 3h, at 37°C, 5% CO2, before imaging.

Compound preparation and testing (day1)

- Prepare dose response curves with 10x concentrations, maintaining constant vehicle solvent concentration

- Prepare negative controls with vehicle solvent (here, 0.1% DMSO)
- Distribute 12.5µL of test compounds or controls per well.

Imaging and data acquisition (day1-3)

- Image 96 well plate at 3h, 6h, 24h and 48h after addition of test compounds

Materials:

- 96-well plate: Greiner Bio-One Black µClear 96-well cell-culture treated plate, ref: 655090
- Nuclear stain: Hoechst 33342 (Invitrogen, ref: H1399)
- Vehicle: DMSO (Fisher BioReagents, ref: BP231-100)
- Culture medium: RPMI 1640 (ATCC modified) (Gibco, ref: A1049101), complemented with 10% FBS and 1% Penicillin/Streptomycin

6. Additional Information

Examples of acquisition channels and of filter settings

- ChromaLive488_Yellow
  - Excitation: 488nm laser OR 475/34nm excitation filter
  - Acquisition: 593/40nm emission filter
- ChromaLive488_Red
  - Excitation: 488nm laser OR 475/34nm excitation filter
  - Acquisition: 692/40nm emission filter
- ChromaLive561
  - Excitation: 561 laser OR 560/32nm excitation filter
  - Acquisition: 593/40nm emission filter
- Optional: DAPI
  - Excitation: 405nm laser OR 377/54nm excitation filter
  - Acquisition: 447/60nm emission filter

7. Control Compound Examples

Cell death mechanism	Control compounds (Concentration range, 1:10 serial dilution)	End-point	Time points
Apoptosis	Actinomycin D (1pM-1µM) Staurosporine (5pM-5 µM)	Actinomycin D: 72h Staurosporine: 24h	12h, 24h, 48h, 72h 3h, 6h, 12h, 24h***
ER stress	Tunicamycin (10pM-10µM) Thapsigargin (1pM-1µM)	Tunicamycin: 24h Thapsigargin: 24h	3h, 6h, 12h, 24h 3h, 6h, 12h, 24h***
Autophagy	Rapamycin (10pM-10µM)	Rapamycin: 72h	12h, 24h, 48h, 72h

Table 2. Doses and duration for MCF-7 cells in 2D

*Only provided as examples. Controls require validation.
**Images could be collected more frequently with the appropriate equipment, especially for time-lapse imaging (controlled temperature and CO_2, auto-focusing, etc.)
***See ChromaLive white paper