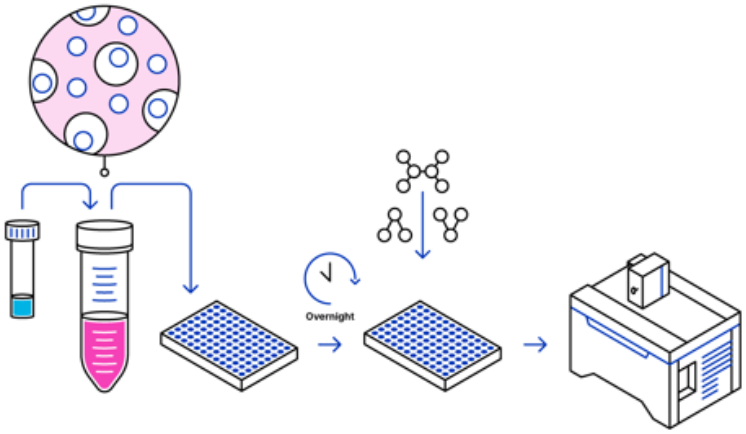
Protocol for ChromaLIVE™ Non-Toxic Dye (Cat. No. 8934)

In Brief

ChromaLIVE™ Non-Toxic Dye is a ready-to-use fluorescent nuclear stain for live cell imaging. It is highly suited for long term 2D and 3D cell organoids imaging, and for monitoring precise tracking of cell proliferation, differentiation, and responses to stimuli over time. Demonstrates excellent cell viability, high signal-to-noise ratios, and robust segmentation in 2D and 3D models.

1. Protocol Overview



2. Content and Storage

Product	Content	Storage	Stability
ChromaLIVE™ Non-Toxic Dye	Diluted in 10 µL of DMSO	-20°C Delivered at room temperature Protect from light	1 year

Table 1. ChromaLIVE™ Non-Toxic Dye Product Information

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

3. General Guidelines

ChromaLIVE™ Non-Toxic Dye dilution and preparation

- Warm up the ChromaLIVE™ Non-Toxic 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™ Non-Toxic Dye in preferred culture medium (1:1000 the provided solution).
- Vortex thoroughly.

Cell culture protocol and compound testing, with ChromaLIVE™ Non-Toxic Dye

- Seed cells at desired density (typically to achieve 70-80% confluence) on imaging support, in previously prepared culture medium with ChromaLIVE™ Non-Toxic Dye.
- Add control compounds, test compounds and negative controls for phenotypes of interest.

NOTE: ChromaLIVE™ Non-Toxic 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™ Non-Toxic Dye staining stabilizes after 12 hours in U2OS cells.

Imaging

NOTE: Keep ChromaLIVE™ Non-Toxic 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™ Non-Toxic Dye needs to be imaged at 2 different wavelengths minimally: ChromaLIVE561, and either ChromaLIVE488_Yellow or ChromaLIVE488_Red as ChromaLIVE488_Yellow and ChromaLIVE488_Red look mostly similar (see figure 3) 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™ Non-Toxic Dye can be imaged at 3 different wavelengths: If possible, both ChromaLIVE488_Yellow and ChromaLIVE488_Red can be used.

ChromaLIVE dye wavelengths:

- ChromaLIVE488_Yellow: excitation at 488nm, image acquisition between 550-630nm
- ChromaLIVE488_Red: excitation at 488nm, image acquisition between 630-750nm
- ChromaLIVE561: excitation at 561nm, image acquisition between 590-630nm

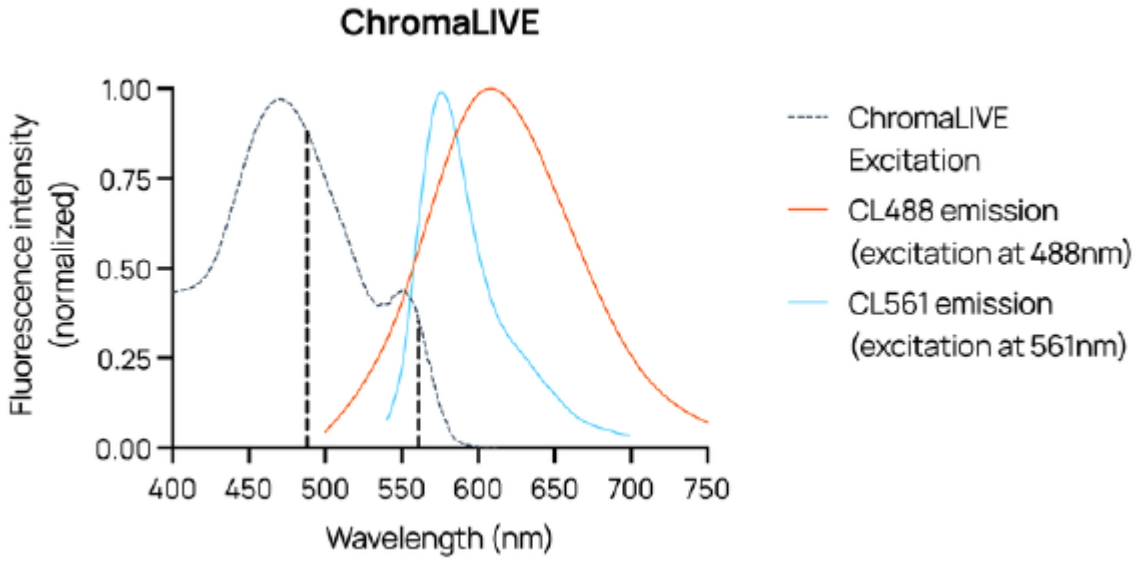


Figure 2: Excitation and emission spectra of ChromaLIVE™ Non-Toxic. Of note: ChromaLIVE™ Non-Toxic is excited at 488nm and 561 nm, with different resulting emission spectra. In green, ChromaLIVE561: emission spectrum when excited around 561nm, in red, ChromaLIVE488: emission spectrum when excited around 488nm.

4. Image Examples

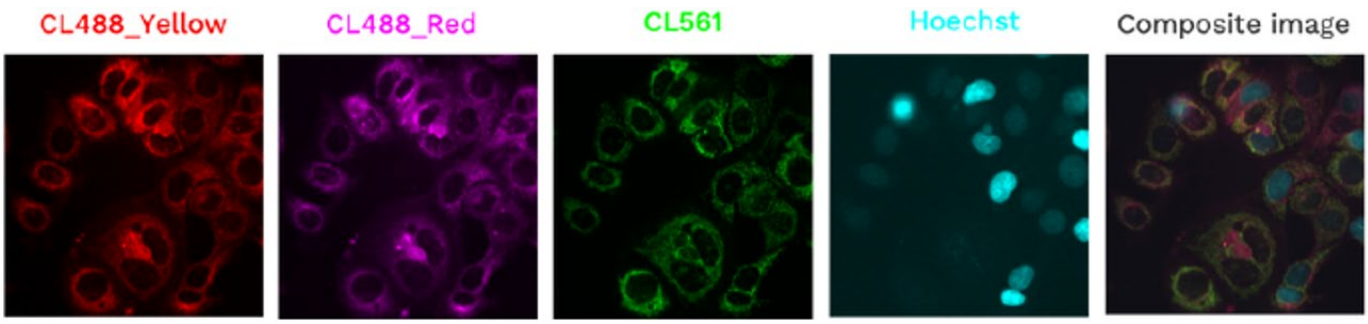


Figure 3. MCF-7 cells stained with ChromaLIVE™ Non-Toxic Dye when seeded (24h before treatment). Images were acquired on Opera QEHS (scale bar not known). Red: ChromaLIVE 488-Yellow, Magenta: ChromaLIVE 488-Red, Green:ChromaLIVE 561-Yellow, Blue: Hoechst 33342.

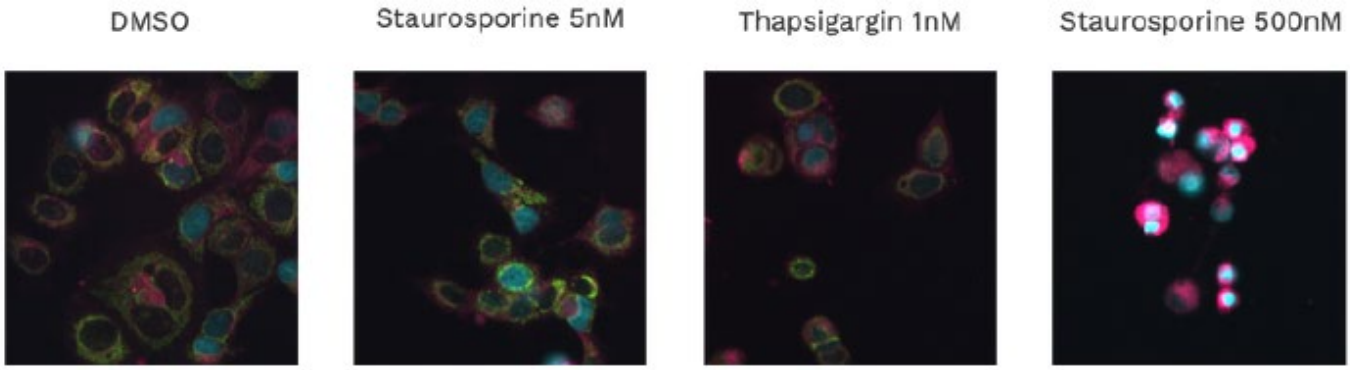


Figure 4. MCF-7 cells with ChromaLIVE at 24h in different conditions. Images are composite images of cells treated for an early apoptotic phenotype (staurosporine 5nM), a stress phenotype (Thapsiargin 1nM), and anapoptotic/necrotic phenotype (staurosporine at 500nM), in comparison with an untreated DMSO control. Red: ChromaLIVE 488-Yellow, Magenta: ChromaLIVE 488-Red, Green: ChromaLIVE 561-Yellow, Blue: Hoechst 33342.

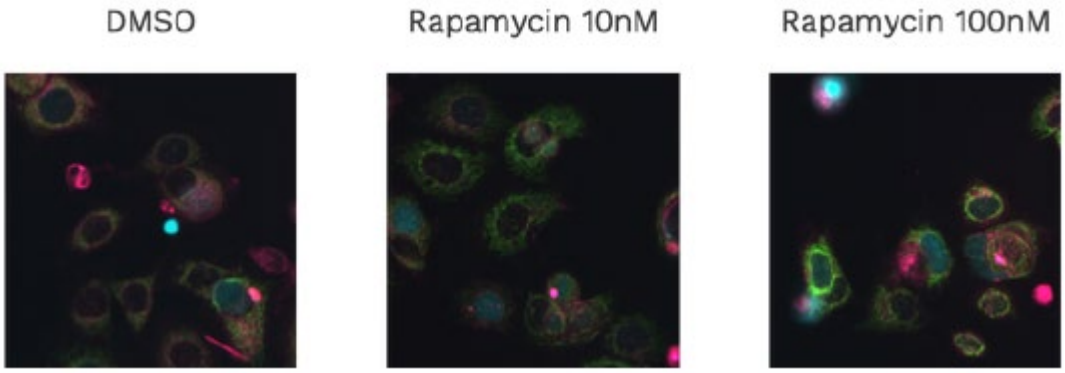


Figure 5. MCF-7 cells with ChromaLIVE at 72h treated with Rapamycin. Images are composite images of cells treated for an autophagy phenotype at 10nM and 100nM of rapamycin, in comparison with an untreated DMSO control. Red: ChromaLIVE 488-Yellow, Magenta: ChromaLIVE 488-Red, Green: ChromaLIVE 561-Yellow, 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™ Non-Toxic Dye dilution and preparation (day0):

- Warm up the ChromaLIVE™ Non-Toxic 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 10 µL ChromaLIVE™ Non-Toxic Dye in 10 mL 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™ Non-Toxic Dye at 80,000 cells/mL.
- Seed 96 well plate with 100 µL cell suspension per well, for a final amount of 8,000 cells per well.
- Incubate overnight at 37°C, 5% CO_2.

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

- Dilute Hoechst 33342 solution at 1µg/mL in culture medium, add 12.5 µL per well for a final concentration of 100 ng/mL.
- Incubate for at least 3h, at 37°C, 5% CO_2, 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 12.5 µL 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.

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 (1 pM-1 µM) Staurosporine (5 pM-5 µM)	Actinomycin D: 72h Staurosporine: 24h	12h, 24h, 48h, 72h 3h, 6h, 12h, 24h
ER stress	Tunicamycin (10 pM-10 µM) Thapsigargin (1 pM-1 µM)	Tunicamycin: 24h Thapsigargin: 24h	3h, 6h, 12h, 24h 3h, 6h, 12h, 24h
Autophagy	Rapamycin (10 pM-10 µM)	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.).