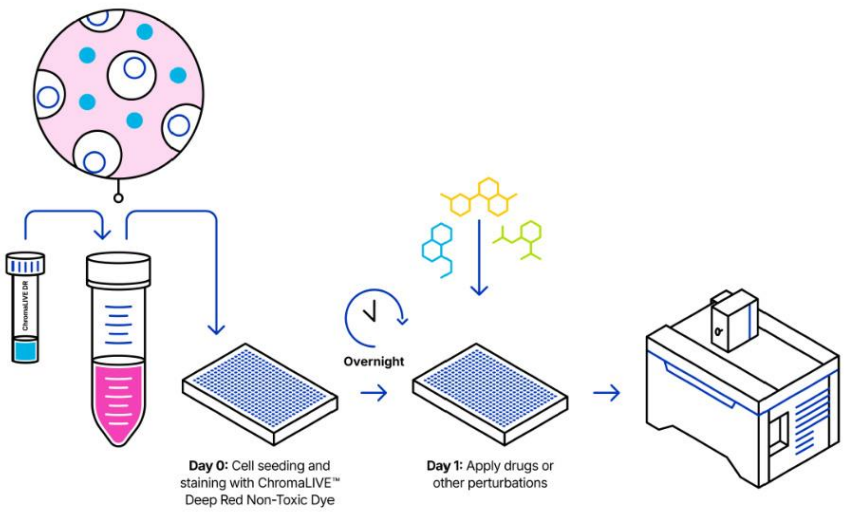
Protocol for ChromaLIVE™ Deep Red Non-Toxic Dye (Cat. No. 9009)

In Brief

ChromaLIVE™ Non-Toxic Deep Red Dye is a ready-to-use fluorescent probe for long term high content phenotypic screening of live cells. Optimized for live cell painting and morphological profiling in 2D monolayers, 3D spheroids or organoids, the dye provides multi organelle read out for unbiased classification of cell states such as proliferation, stress responses, autophagy, apoptosis or other phenotypic transitions. ChromaLIVE™ Deep Red Non-Toxic Dye can be used with NucleoLIVE™ Non-Toxic Dye (Cat. No. 8935) to get even more insight from live cell painting assays.

ChromaLIVE™ is a trademark of Saguaro Biosciences.

1. Protocol Overview



2. Content and Storage

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

Table 1. ChromaLIVE™ Deep Red Non-Toxic Dye Product Information

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

3. General Guidelines

ChromaLIVE™ Deep Red Non-Toxic Dye Dilution and Preparation

- Warm up the ChromaLIVE™ Deep Red 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 Deep Red Dye 1,000-fold in preferred cell culture medium
- Vortex thoroughly
- Seed cells at desired density (typically to achieve 70-80% confluence) in cell culture medium containing ChromaLIVE™ Deep Red Dye in a black multi-well plate. Return to the incubator at 37°C, 5% CO_2 overnight
- No washing step is required prior to imaging. Keep ChromaLIVE™ Deep Red in solution throughout the assay

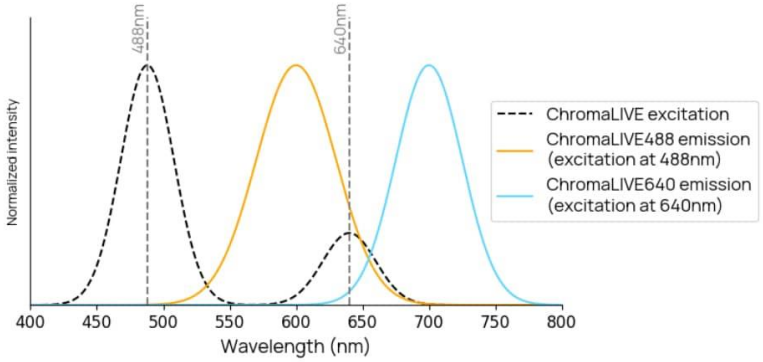
Alternative Cell Culture Indications for ChromaLIVE™ Deep Red Non-Toxic Dye

- While we recommend seeding cells in the presence of diluted ChromaLIVE™ Deep Red, the dye can be added after cell seeding, before or following compound addition. Optimization of seeding density and incubation time prior to imaging are required. For reference, ChromaLIVE™ Deep Red staining stabilizes after 12 hours in U2OS cells
- A nuclear dye can be added to allow cell segmentation during data analysis, such as the NucleoLIVE™ Non-Toxic Dye (Cat. No. 8935). We recommend running a preliminary imaging test on cells treated with single dyes to validate the staining kinetics and absence of fluorescence bleed-through between the nuclear dye and ChromaLIVE™ Deep Red channels on your system

Imaging Parameters

- Two wavelengths (Recommended): ChromaLIVE™ Deep Red dye needs to be imaged at two different wavelengths minimally: ChromaLIVE640, and either ChromaLIVE488_Yellow or ChromaLIVE488_Red. Selecting only one of the two CL488 channels is sufficient for differentiating between cellular phenotypes, even subtle ones
- Three wavelengths (Optional): While ChromaLIVE488_Yellow and ChromaLIVE488_Red look mostly similar (see **Figure 2**), they can still provide slightly different information. When feasible, acquiring both ChromaLIVE488 channels is recommended to maximize data richness. However, this approach comes with increased acquisition time and larger file sizes, which should be taken into consideration

4. Technical Specifications & Instrument compatibility



Channel and acquisition parameters
ChromaLIVE488_Yellow Excitation:488nm / Emission: 550-630nm
ChromaLIVE488_Red Excitation: 488nm / Emission: 630-750nm
ChromaLIVE640 Excitation: 640nm / Emission: 665-705nm

Figure 1. Excitation and emission spectra of ChromaLIVE™ Deep Red Non-Toxic Dye. ChromaLIVE™ is excited at 488nm and 640nm, with different resulting emission spectra. In orange, ChromaLIVE488 emission when excited around 488nm. In cyan, ChromaLIVE640 emission spectrum when excited around 640nm.

Instrument compatibility

Manufacturer	Instrument	Settings	Filters	Mode
Molecular Devices	ImageXpress Confocal	ChromaLIVE488_Yellow	Cyan / FITC / Cy3	Widefield / Confocal
	ImageXpress Confocal HT.ai	ChromaLIVE488_Red	Cyan / FITC / Cy5	Widefield / Confocal
		ChromaLIVE640	Red / Cy5 / Cy5	Widefield / Confocal
PerkinElmer / Revvity	Opera Phenix	ChromaLIVE488_Yellow	488 / 570-630	Widefield only
	Opera Phenix Plus	ChromaLIVE488_Red	488 / 650-760	Widefield / Confocal
		ChromaLIVE640	640 / 650-760	Widefield / Confocal
	Operetta CLS	ChromaLIVE488_Yellow	460-490 / 570-650	Widefield only
		ChromaLIVE488_Red	460-490 / 655-760	Widefield / Confocal
		ChromaLIVE640	615-645 / 655-760	Widefield / Confocal
Yokogawa	CQ1	ChromaLIVE488_Yellow	488 / 617/73	Confocal
		ChromaLIVE488_Red	488 / 685/40	Confocal
		ChromaLIVE640	640 / 685/40	Confocal
	CV8000	ChromaLIVE488_Yellow	488 / 600/37	Confocal
		ChromaLIVE488_Red	488 / 676/29	Confocal
		ChromaLIVE640	640 / 676/29	Confocal

* ChromaLIVE™ Deep Red is compatible with other high-content imagers and confocal microscopes. Please refer to **Figure 1** for technical specifications.

4.1 Image Examples and Recommended Positive Control Compounds*

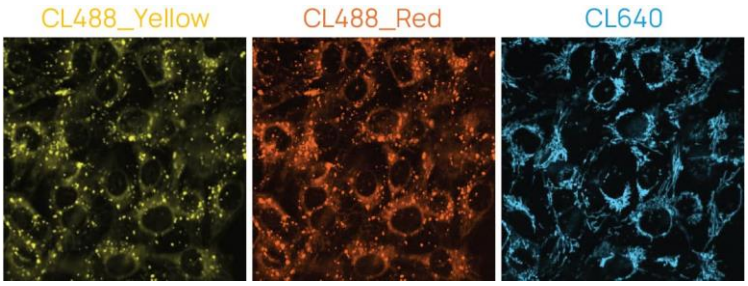


Figure 2. MCF-7 cells stained with ChromaLIVE™ Deep Red. Yellow: ChromaLIVE488_Yellow, Orange: ChromaLIVE488_Red, Cyan: ChromaLIVE640.

Table 2. Doses and treatment durations for MCF7 cells in 2D. Bold represents recommended assay endpoint

Cell Death Mechanism	Apoptosis	ER Stress	Autophagy
Control Compound Concentration Range (1:10, serial dilution) and Timepoints **	Actinomycin D (1 pM-1 µM) 12h, 24h, 48h, **72h** Staurosporine (5 pM-5 µM) 3h, 6h, 12h, **24h*****	Tunicamycin (10 pM-10 µM) 3h, 6h, 12h, **24h** Thapsigargin (1 pM-1 µM) 3h, 6h, 12h, **24h*****	Rapamycin (10 pM-10 µM) 12h, 24h, 48h, **72h**

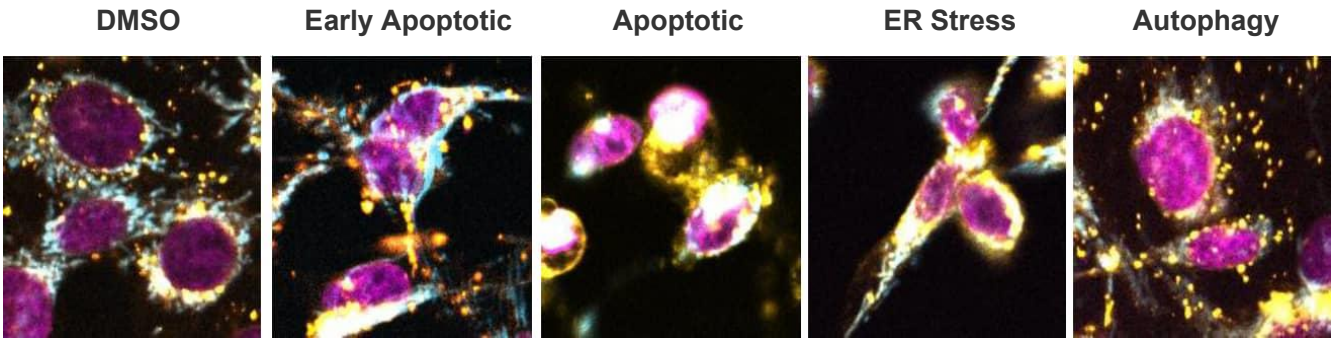


Figure 3. MCF-7 cells with ChromaLIVE™ Deep Red Non-Toxic Dye and NucleoLIVE™ Non-Toxic Dye (Cat. No. 8935). Cells were treated for an early apoptotic or apoptotic phenotype (staurosporine 5 nM and 500 nM for 24h, respectively), an ER stress (thapsigargin 1 nM, 24h) or an autophagy phenotype (rapamycin 100 nM, 72h). Magenta: NucleoLIVE™, Yellow:CL488_Yellow, Orange: CL488_Red, Cyan: CL640.

* Compounds provided as examples only. Validation required for each experimental protocol.

** Images could be collected more frequently with the appropriate equipment, especially for time-lapse imaging (controlled temperature and CO_2, auto-focusing, etc.)

4.2. Example Protocol (for kinetic, 2D live-cell assay)

MCF7 cells treated with standard compounds for apoptosis, ER stress and autophagy. MCF7 are cultured in RPMI 1640 complemented with 10% FBS and 1% Penicillin/Streptomycin.

ChromaLIVE™ Deep Red and NucleoLIVE™ Non-Toxic Dye Dilution and Preparation (Day 0):

• Warm up the ChromaLIVE™ and NucleoLIVE™ dye tubes to room temperature before use and gently spin to collect any dye solution that may remain near the cap
• Dilute 10 µL ChromaLIVE™ Deep Red dye in 10 mL culture medium (1000-fold)
• Dilute 10 µL NucleoLIVE™ dye in the same 10 mL culture medium (1000-fold)
• Vortex thoroughly

Cell Culture Protocol with ChromaLIVE™ Deep Red (Day 0):

• Harvest and count MCF7 cells
• Resuspend cells in prepared culture medium with ChromaLIVE™ Deep and NucleoLIVE™ dyes at 80,000 cells/mL
• Seed 96-well plate with 100µL cell suspension per well to a final density of 8,000 cells per well
• Incubate overnight at 37°C, 5% CO_2

Standard Compound Preparation and Addition (Day 1):

• Prepare dose-response curves with 10x concentrations, maintaining constant vehicle (0.1% DMSO) solvent concentration
• Prepare negative controls with 0.1% DMSO in complete media
• Distribute 12.5 µL of test compounds or controls per well

Imaging and Data Acquisition (Days 1-3):

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