Protocol for ChromaLIVE™ Deep Red + ER-LIVE™ Dyes

In Brief

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

ER-LIVE™ Endoplasmic Reticulum Dye (Cat. No. 9010) is a ready-to-use fluorescent probe for long-term imaging of ER in live cells. ER-LIVE™ Endoplasmic Reticulum Dye can be multiplexed with ChromaLIVE Deep Red Non-Toxic Dye (Cat. No. 9009) for phenotypic screening.

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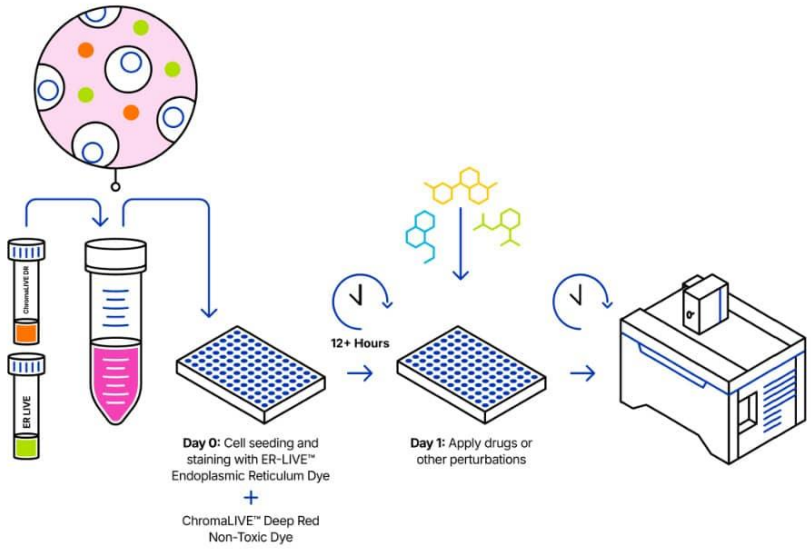
1. Protocol Overview



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
ER-LIVE™ Non-Toxic Dye	Diluted in 50 µL of DMSO		

Table 1. ChromaLIVE™ + ER-LIVE™ Dyes Product Information
Intended Use: For research use only. Not for use in diagnostics or therapeutic procedures.

3. General Guidelines

ChromaLIVE™ + ER-LIVE™ Dyes Dilution and Preparation

- Warm up the ChromaLIVE™ and ER-LIVE™ tubes to room temperature to avoid condensation to form and water to get into the anhydrous dye solution
- Gently spin the tubes before use to collect any dye solution that may remain near the cap
- Dilute ChromaLIVE™ and ER-LIVE™ 1,000-fold each in preferred cell culture medium. For 10 mL of media, add 10 µL of ChromaLIVE™ and 10 µL of ER-LIVE™
- Vortex thoroughly
  - A nuclear dye can be added to allow cell segmentation during data analysis, such as 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™ + ER-LIVE™ channels on your system.
  - Note: We recommend 1X as a starting point for optimization. Higher or lower concentrations may be optimal for different imaging systems and cell models.
- Seed cells at desired density (typically to achieve 70-80% confluence) in cell culture medium containing ChromaLIVE™ + ER-LIVE™ mix 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™ + ER-LIVE™ mix in solution throughout the assay

Alternative Cell Culture Indications for ChromaLIVE™ + ER-LIVE™

- While we recommend seeding cells in the presence of diluted ChromaLIVE™ + ER-LIVE™, the mix can be added after cell seeding, before or following compound addition. Optimisation of seeding density and incubation times prior to imaging are required. For reference, ChromaLIVE™ + ER-LIVE™ staining stabilizes after 12 hours in U2OS cells

Imaging Parameters for ER-LIVE™

- Three wavelengths (Recommended): ChromaLIVE™ + ER-LIVE™ dyes need to be imaged at three different wavelengths minimally: ChromaLIVE488_Green, ChromaLIVE640, and ChromaLIVE488_Red
- Four 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_Red and Yellow 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

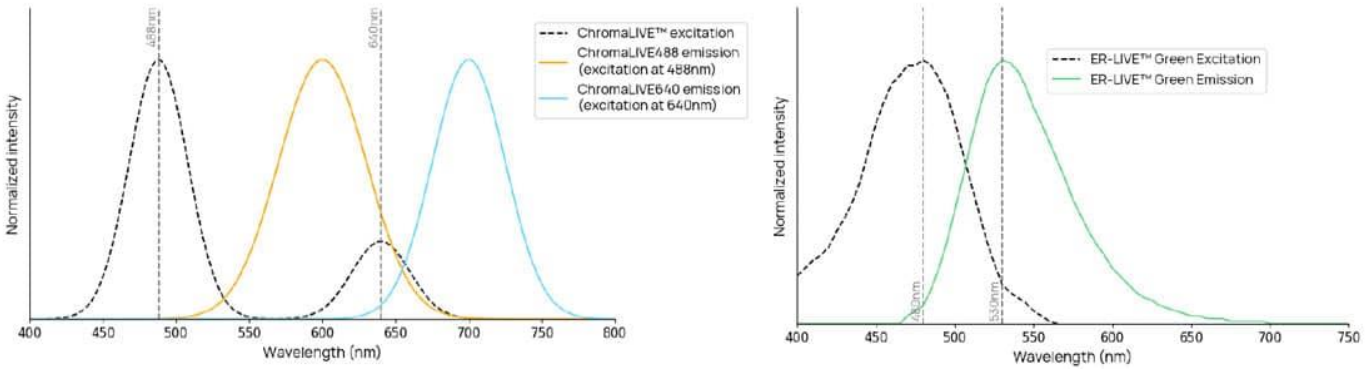


Figure 1. Excitation and emission spectra. ChromaLIVE™ is excited at 488nm and 640nm, with different resulting emission spectra. In orange, ChromaLIVE488 emission when excited around 488nm. In cyan, ChromaLIVE640 emission spectrum when excited around 640nm. ER-LIVE™ is excited at 488nm and emits around 530nm.

Table 2. Channels and general acquisition parameters

Channels and general acquisition parameters			
ChromaLIVE488_Green Excitation: 488nm Emission: 500-550 nm	**ChromaLIVE488_Yellow** Excitation: 488nm Emission: 550-630 nm	**ChromaLIVE488_Red** Excitation: 488nm Emission: 630-750 nm	**ChromaLIVE640** Excitation: 640nm Emission: 665-705 nm

Table 3. Instrument compatibility*

Manufacturer	Instrument	Settings	Filters	Mode
PerkinElmer/ Revvity	Opera Phenix	ER-LIVE	488/ 500	Widefield / Confocal
	Opera Phenix Plus	ChromaLIVE488_Yellow	488 / 570-630	
		ChromaLIVE488_Red	488 / 650-760	
		ChromaLIVE640	640 / 650-760	
	Operetta CLS	ER-LIVE	460-490 / 500-550	
		ChromaLIVE488_Yellow	460-490 / 570-650	
		ChromaLIVE488_Red	460-490 / 655-760	
		ChromaLIVE640	615-645 / 655-760	
Yokogawa	CQ1	ER-LIVE	488 / 525-550	Confocal
		ChromaLIVE488_Yellow	488 / 617-673	
		ChromaLIVE488_Red	488 / 685-640	
		ChromaLIVE640	640 / 685-640	
	CV8000	ER-LIVE	488 / 525-550	
		ChromaLIVE488_Yellow	488 / 600-637	
		ChromaLIVE488_Red	488 / 676/29	
		ChromaLIVE640	640 / 676/29	
Molecular Devices	ImageXpress Confocal	ChromaLIVE488_Yellow + ER-LIVE**	Cyan / FITC / Cy3	Widefield / Confocal
	ImageXpress Confocal HT.ai	ChromaLIVE488_Red	Cyan / FITC / Cy5	
		ChromaLIVE640	Red / Cy5 / Cy5	

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

** On some instruments, the ChromaLIVE488_Yellow and ChromaLIVE488_Green (ER-LIVE) channels will be acquired in the same channel.

5. Image Examples and Performance Comparison to ChromaLIVE™ & Hoechst

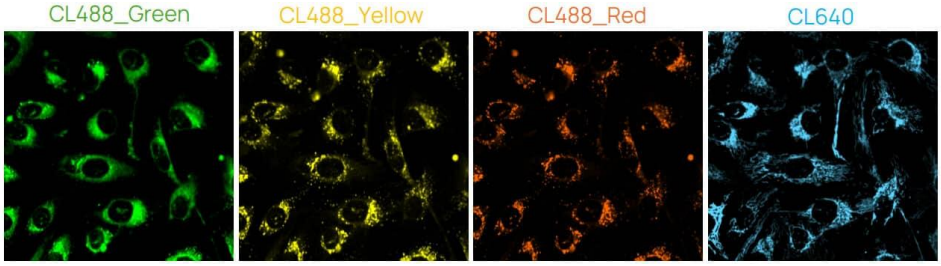


Figure 2. U2OS cells stained with ChromaLIVE™ + ER-LIVE™. Green: ChromaLIVE488_Green, Yellow ChromaLIVE488_Yellow, Orange: ChromaLIVE488_Red, Cyan: ChromaLIVE640.

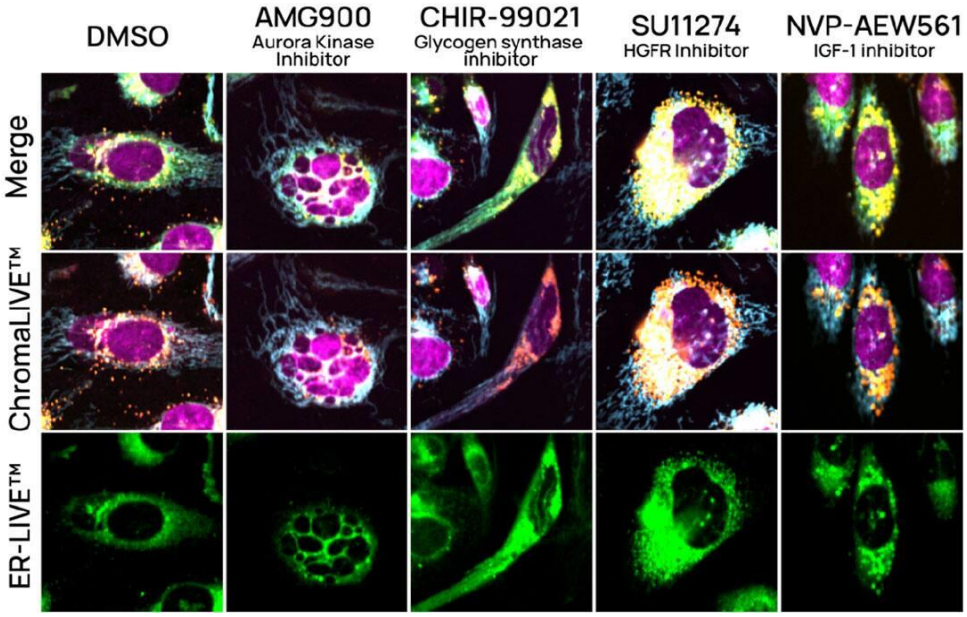


Figure 3. U2OS cells with ChromaLIVE™ + ER-LIVE™ and NucleoLIVE™. Representative images at 48h for vehicle control (0.1% DMSO), AMG900 (aurora kinase inhibitor), CHIR-99021 (Glygocen synthase inhibitor), SU1124 (HGFR inhibitor) and NVP-AEW561 (IFG-1 inhibitor). Green: ChromaLIVE488_Green, Yellow: ChromaLIVE488_Yellow, Orange: ChromaLIVE488_Red, Cyan: ChromaLIVE640, Magenta: CL561 (NucleoLIVE™).

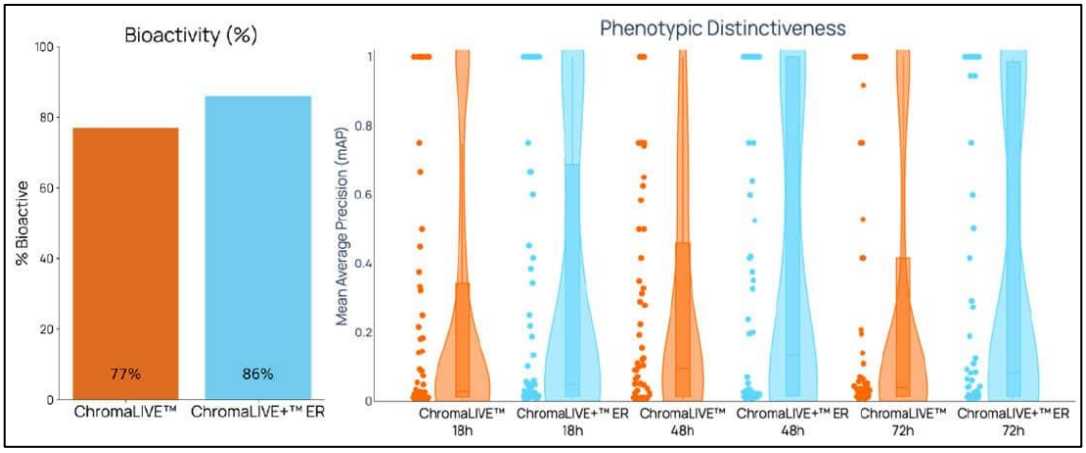


Figure 4. ChromaLIVE™ + ER-LIVE™ improves compound bioactivity detection and phenotypic distinctiveness compared to ChromaLIVE™. U2OS cells were stained with ChromaLIVE™ and Hoechst (100 ng/mL, orange) or ChromaLIVE™ + ER-LIVE™ and NucleoLIVE™ (cyan). **A)** Morphological profiling with ChromaLIVE™ + ER-LIVE™ and NucleoLIVE™ improved bioactivity detection by 9% for the 90 compounds of the JUMP-MOA plate (average from independent experiments). **B)** Additionally, ChromaLIVE™ + ER-LIVE™ improved phenotypic distinctiveness (i.e higher distinctiveness indicates more unique morphological signatures between compounds) at several timepoints of the assay.

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

MCF7 cells are 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™ + ER-LIVE™ and NucleoLIVE™ Dye Dilution and Preparation (Day 0):

- Warm up the ChromaLIVE™, ER-LIVE™ 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 of ChromaLIVE™, 10 µL of ER-LIVE™ and 10 µL of NucleoLIVE™ in 10 mL culture medium (1000-fold)
- Vortex thoroughly

Cell Culture Protocol with ChromaLIVE™ + ER-LIVE™ (Day 0):

- Harvest and count MCF7 cells
- Resuspend cells in prepared culture medium with ChromaLIVE™ + ER-LIVE™ mix and NucleoLIVE™ at 80,000 cells/mL
- Seed 96-well plate with 100 µL 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

7. Recommended Positive Control Compounds for Cell Death Mechanisms*

Table 4. 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**

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

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