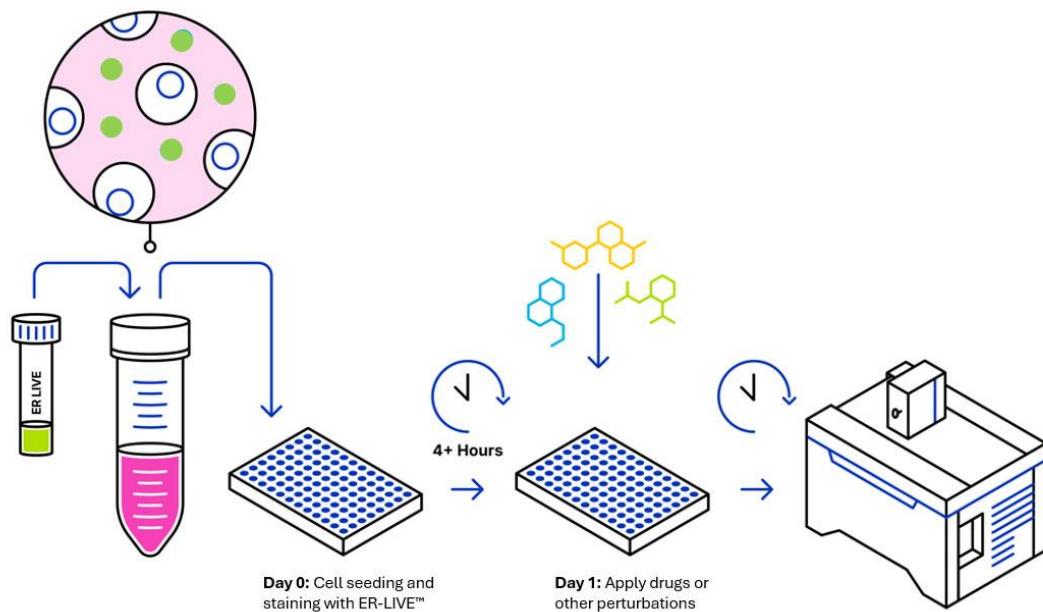


Protocol for ER-LIVE™ Endoplasmic Reticulum Dye

In Brief

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.

1. Protocol Overview



2. Content and Storage

| Product | Content | Storage | Stability |
|------------------------------------|--------------------------|--|-----------|
| ER-LIVE™ Endoplasmic Reticulum Dye | Diluted in 50 µL of DMSO | -20°C Delivered at room temperature Protect from light | 1 year |

Table 1. ER-LIVE™ dye Product Information

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

3. General Guidelines

ER-LIVE™ dye Dilution and Preparation

- Warm up the ER-LIVE™ 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 ER-LIVE™ dye 1,000-fold in preferred cell culture medium
- Vortex thoroughly
 - 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 ER-LIVE™ dye in a black multi-well plate. Return to the incubator at 37°C, 5% CO₂ overnight
- No washing step is required prior to imaging. Keep ER-LIVE™ in solution throughout the assay

Alternative Cell Culture Indications for ER-LIVE™

- While we recommend seeding cells in the presence of diluted ER-LIVE™, the dye 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, ER-LIVE™ staining stabilizes after 4 hours in U2OS cells (at a concentration of 1X)
- 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 ER-LIVE™ channels on your system

4. Technical Specifications & Instrument Compatibility

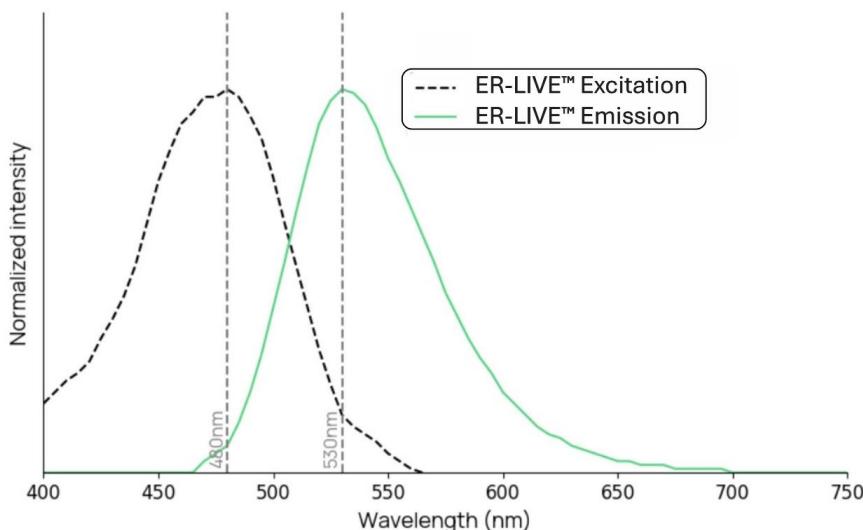


Figure 1. Excitation and emission spectra. ER-LIVE™ is excited at 480nm and emits around 530nm.

Table 2. Instrument compatibility of ER-LIVE™

| Manufacturer | Instrument | Filters | Mode |
|----------------------|----------------------------|-------------------|---------------------|
| Molecular Devices | ImageXpress Confocal | Cyan / FITC / Cy3 | Widefield /Confocal |
| | ImageXpress Confocal HT.ai | | |
| PerkinElmer/ Revvity | Opera Phenix | 488/ 500-550 | Widefield only |
| | Opera Phenix Plus | | Widefield /Confocal |
| | Operetta CLS | 460-490 / 500-550 | Widefield /Confocal |
| Yokogawa | CQ1 | 488 / 525-550 | Confocal |
| | CV8000 | 488 / 525-550 | |

* ER-LIVE™ is compatible with other high-content imagers and confocal microscopes.

Please refer to **Figure.1** for technical specifications.

5. ER-LIVE™ non-toxic dye, allowing for high quality staining without compromising cell health

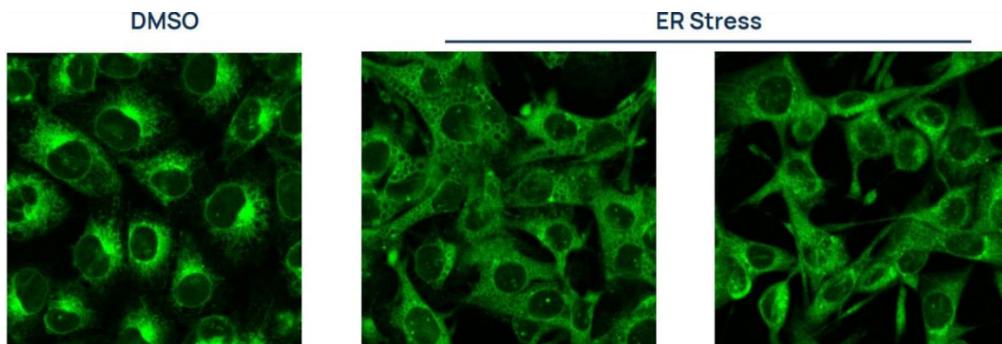


Figure 2. MCF7 cells stained with ER-LIVE™. Composite images of cells undergoing ER stress following Thapsigargin treatment for 24h (middle: 1nM; right: 50nM) compared to an untreated DMSO control. Recommended positive control compounds and treatment durations to induce ER stress (for MCF7 cells). **Bold** represents recommended assay endpoint.

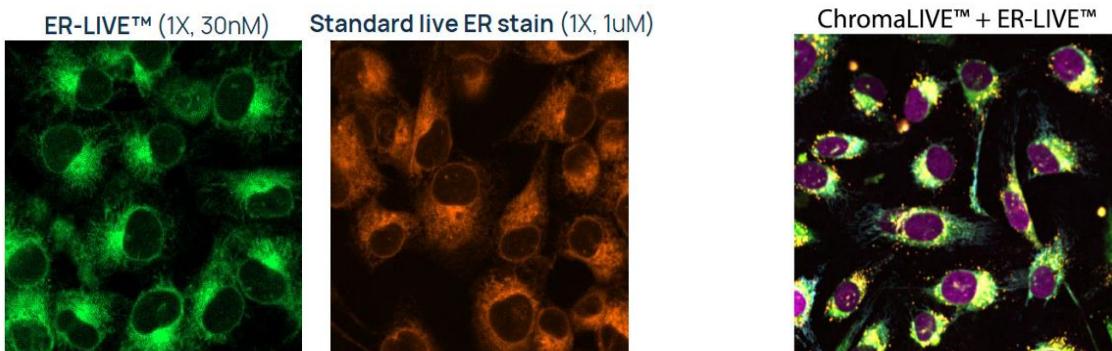


Figure 3. ER-LIVE™ delivers bright, stable endoplasmic reticulum labeling at nanomolar concentrations, minimizing disruption of native cell biology. MCF7 cells stained with ER-LIVE™ (green) or a standard live ER stain (orange) for 24 hours. ER-LIVE™ provides comparable brightness at much lower concentrations, and its mix-and-read formulation allows continuous use in culture for extended imaging or kinetic studies without perturbing cell physiology.

Figure 4. ER-LIVE™ can be multiplexed with ChromaLIVE™ and NucleoLIVE™ to gain deeper biological insights. U2OS cells stained with ChromaLIVE™ (Orange: CL488_Red, Cyan: CL640), ER-LIVE™ (green) and NucleoLIVE™ (magenta, CL561).

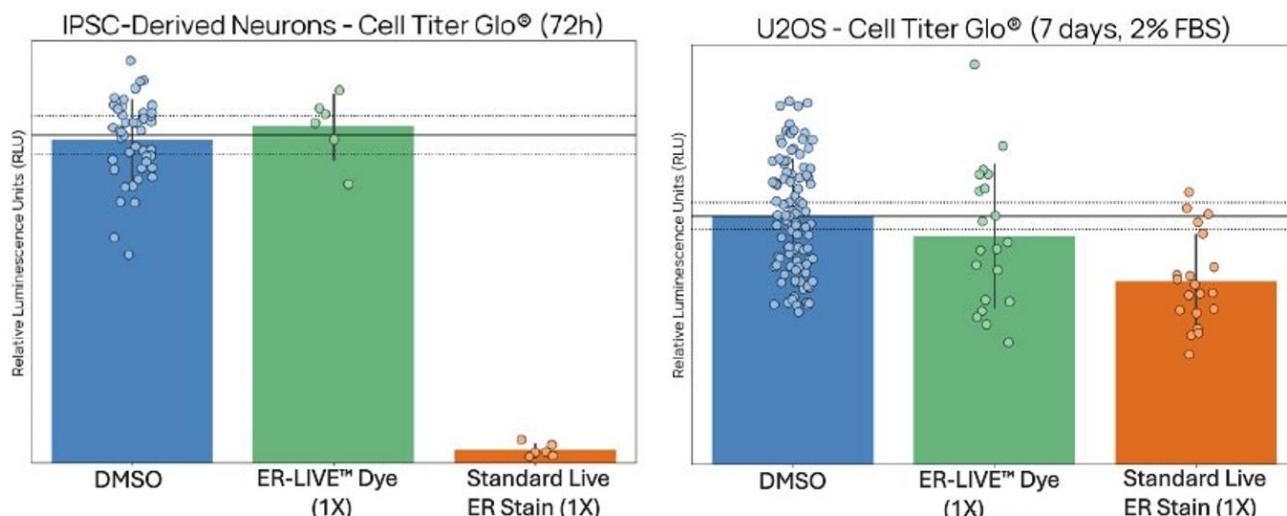


Figure 5. ER-LIVE™ preserves normal cell proliferation and viability, even in sensitive models. iPSC-derived neurons and U2OS cells (maintained in 2% FBS for increased sensitivity) showed comparable viability to DMSO controls after 24-hour incubation with ER-LIVE™. In contrast, the standard live ER dye significantly reduced cell health - demonstrating ER-LIVE™'s non-toxic, non-disruptive profile, enabling extended live-cell imaging without compromising cellular physiology. Both dyes were used at their respective recommended 1X concentrations.

6. Recommended Positive Control Compounds for ER stress*

Table 3. Doses and treatment durations for MCF7 cells in 2D. Bold represents recommended assay endpoint.

| Control Compound | Tunicamycin | Thapsigargin |
|--|--|--------------------------------------|
| Concentration Range (1:10, serial dilution) and Timepoints ** | 10 pM-10 µM 3h, 6h, 12h, 24h | 1 pM-1 µM 3h, 6h, 12h, 24h |

* 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 CO₂, auto-focusing, etc.)

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

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

ER-LIVE™ Dye Dilution and Preparation (Day 0):

- Warm up the ER-LIVE™ dye tube to room temperature before use and gently spin to collect any dye solution that may remain near the cap
- Dilute 10 µL of ChromaLIVE™ dye in 10 mL culture medium (1000-fold)
- Optional: Dilute 10 µL NucleoLIVE™ dye in the same 10 mL culture medium (1000-fold)
- Vortex thoroughly

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

- Harvest and count MCF7 cells
- Resuspend cells in prepared culture medium with ER-LIVE™ (and NucleoLIVE™, if applicable) 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% CO₂

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 after addition of test compounds

ER-LIVE™, NucleoLIVE™, and ChromaLIVE™ are trademarks of Saguaro Biosciences.