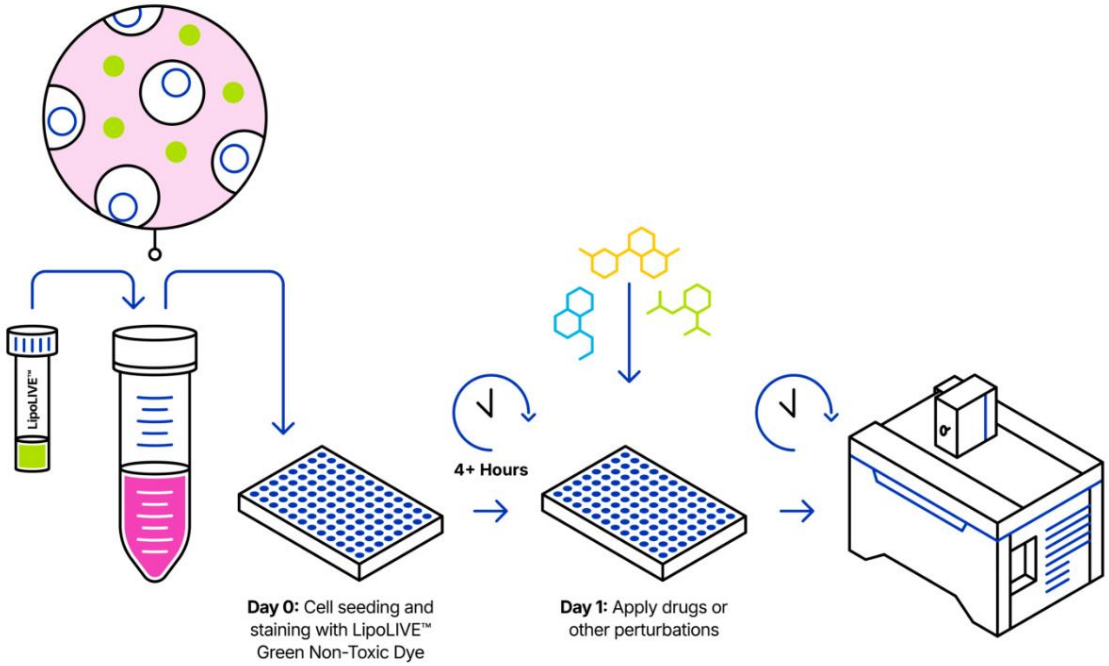
Protocol for LipoLIVE™ Green Non-Toxic Dye (Cat. No. 9026)

In Brief

LipoLIVE™ Green Non-Toxic Dye is a ready-to-use fluorescent probe for the real-time visualization of lipid droplet and neutral lipids in live cells. It does not require cell fixation or affect cell health and maintains the physiological integrity of models, enabling free-wash monitoring of lipid synthesis, storage, and mobilization.

LipoLIVE™ is a trademark of Saguaro Biosciences.

1. Protocol Overview



2. Content and Storage

Product	Content	Storage	Stability
LipoLIVE™ Green Non-Toxic Dye	Diluted in 50 µL of DMSO	-20°C Delivered at room temperature Protect from light	1 year

Table 1. LipoLIVE™ Green Non-Toxic Dye Product Information

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

3. General Guidelines

LipoLIVE™ Green Non-Toxic Dye dilution and preparation

- Warm up the LipoLIVE™ Green 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 LipoLIVE™ Green Non-Toxic Dye 1,000-fold in preferred 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 LipoLIVE™ Green Non-Toxic 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 LipoLIVE™ Green Non-Toxic Dye in solution throughout the assay.

Alternative Cell Culture Indications for LipoLIVE™ Green Non-Toxic Dye

- While we recommend seeding cells in the presence of diluted LipoLIVE™ Green Non-Toxic Dye, the dye can be added after cell seeding, before or following compound addition. Optimisation of seeding density and incubation prior to imaging are required. For reference, LipoLIVE™ Green Non-Toxic Dye 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) or NucleoLIVE™ Blue Non-Toxic Dye (Cat. No. 9011). 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 LipoLIVE™ Green channels on your system.

4. Technical Specifications & Instrument compatibility

LipoLIVE™ Green Non-Toxic Dye is a green fluorescent dye that is compatible with GFP, Cy3 or FITC filters.

Instrument compatibility of LipoLIVE™ Green Non-Toxic Dye

Manufacturer	Instrument	Filters	Mode
Molecular Devices	ImageXpress Confocal ImageXpress Confocal HT.ai	Cyan / FITC / Cy3	Widefield / Confocal
PerkinElmer / Revvity	Opera Phenix Opera Phenix Plus Operetta CLS	488 / 500-550 460/90 / 500/50	Widefield only Widefield / Confocal Widefield / Confocal
Yokogawa	CQ1	488 / 525/50	Confocal
	CV8000	488 / 525/50	Confocal

LipoLIVE™ Green Non-Toxic Dye is compatible with other high-content imagers and confocal microscopes.

5. LipoLIVE™ Green Non-Toxic Dye, allowing for high quality staining of lipid droplets and neutral lipids without compromising cell health

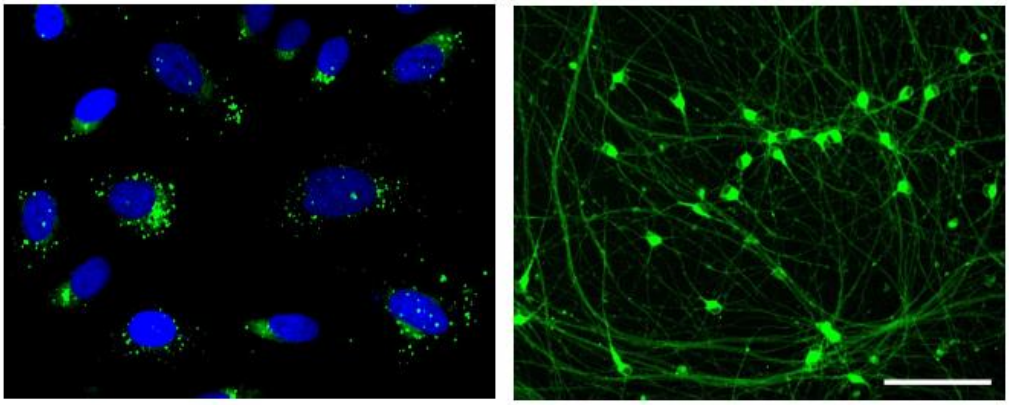


Figure 2. U2OS cells (left) and iPSC-derived neurons (right) stained with LipoLIVE™ Green Non-Toxic Dye. Composite images of cells stained LipoLIVE™ Green Non-Toxic Dye for 72h.

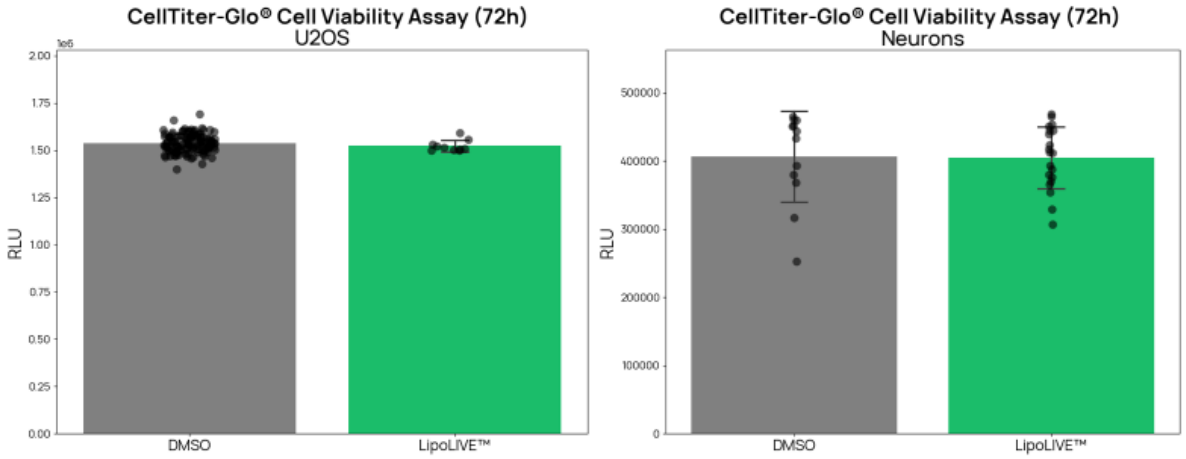


Figure 3. LIPOLIVE™ Green Non-Toxic Dye preserves normal cell proliferation and viability, even in sensitive models. U2OS cells and iPSC-derived neurons showed comparable viability to DMSO controls after 72-hour incubation with LIPOLIVE™ Green Non-Toxic Dye.

6. Recommended Positive Control Compounds for Lipid Studies*

Table 2. Compounds and concentration for U2OS cells in 2D.

Compound	Mechanism	Concentrations
Oleic Acid	Long-chain monounsaturated fatty, stimulating the rapid formation of cytosolic lipid droplets.	100 nM-10 µM
Palmitic Acid	Long-chain saturated fatty acid, inducing lipotoxic stress and the formation of small clustered cytosolic lipid droplets.	5 µM-500 µM

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

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

U2OS cells are treated with standard compounds for ER stress and apoptosis. MCF7 are cultured in DMEM/F-12 complemented with 10% FBS and 1% Penicillin/Streptomycin.

LipoLIVE™ Green Non-Toxic Dye Dilution and Preparation (Day 0):

- Warm up the LipoLIVE™ Green Non-Toxic Dye tubes to room temperature before use and gently spin to collect any dye solution that may remain near the cap.
- Optional: Dilute 10 µL NucleoLIVE™ Non-Toxic Dye in the same 10 mL culture medium (1000-fold).
- Vortex thoroughly.

Cell Culture Protocol with LipoLIVE™ Green Non-toxic Dye (Day 0):

- Harvest and count MCF7 cells.
- Resuspend cells in prepared culture medium with LipoLIVE™ Green Non-Toxic Dye (and NucleoLIVE™ Non-Toxic Dye, 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% 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 and 24h after addition of test compounds.