

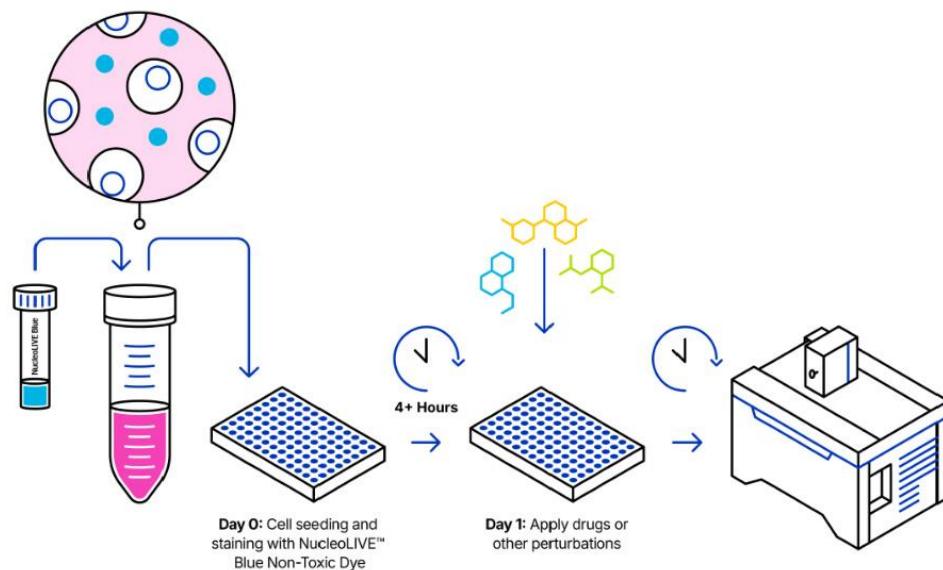
## Protocol for NucleoLIVE™ Blue Non-Toxic Dye (Cat. No. 9011)

### In Brief

NucleoLIVE™ Blue Non-Toxic Dye is a ready-to-use fluorescent probe for long term high content nuclear imaging in live cells. NucleoLIVE™ Blue, is the same as the original NucleoLIVE™ Non-Toxic Dye (Red), but with different spectral properties, allowing compatibility with ChromaLIVE™ Non-Toxic Dye (Cat. No.8934). Ideal for long term 2D and 3D cell organoids high-content imaging and real-time cell viability assays. NucleoLIVE™ Blue Non-Toxic Dye facilitates cell counting to accurately quantify cell toxicity, viability, and morphology by fluorescence on the blue channel, and can be used to generate growth curves to quantify drug effects on cell proliferation in real time and over multiple days. Allow wash-free imaging. NucleoLIVE™ Blue Non-Toxic Dye is provided as 50 µL of 1000x concentrate in DMSO.

*NucleoLIVE™ is a trademark of Saguaro Biosciences.*

### 1. Protocol Overview



### 2. Content and Storage

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

**Table 1.** NucleoLIVE™ Blue Non-Toxic Dye Product Information

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

### 3. General Guidelines

#### NucleoLIVE™ Blue Non-Toxic Dye Dilution and Preparation

- Warm up the NucleoLIVE™ Blue 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 NucleoLIVE™ Blue Non-Toxic 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 NucleoLIVE™ Blue Non-Toxic Dye in a black multi-well plate. Return to the incubator at 37°C, 5% CO<sub>2</sub> for at least 4 hours
- No washing step is required prior to imaging. Keep NucleoLIVE™ Blue Non-Toxic Dye in solution throughout the assay

#### Alternative Cell Culture Indications for NucleoLIVE™ Blue Non-Toxic Dye

- While we recommend seeding cells in the presence of diluted NucleoLIVE™ Blue Non-Toxic Dye, the dye can be added after cell seeding, before or following compound addition. Optimization of seeding density and incubation times prior to imaging are required. For reference, NucleoLIVE™ Blue Non-Toxic Dye staining stabilizes after 4 hours in MCF-7 cells

#### Notes

- Probe concentration and incubation times are given as general guidelines and have been validated on MCF-7 breast cancer cells:
  - (1) Higher concentrations (1:500 dilution) can be used for harder-to-stain cell lines, or shorter labeling periods. Lower concentrations (1:2,000-5,000 dilutions) can be used when imaging live cells for longer periods of time (>24h)
  - (2) Incubation times with the probe should be validated for each cell line, especially when using higher dilutions.

