


LIVE/DEAD™ Viability/Cytotoxicity Assay Kit

Two-color (green/deep red) fluorescent cell viability assay

Catalog Numbers L32250

Pub. No. MAN0025668 Rev. A.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

The LIVE/DEAD™ Viability/Cytotoxicity Assay Kit (green/deep red) with Calcein, AM and SYTOX™ Deep Red Nucleic Acid Stain is a two-color fluorescent cell viability assay that is based on the simultaneous determination of live and dead cells. After a brief incubation with the stain, live cells fluoresce an intense uniform green, while dead cells fluoresce deep red/far red. Cellular viability is determined by counting cells using standard filter sets, FITC/GFP (live cells) and deep red/Cy5™ (dead cells). The assay can be multiplexed with red fluorescent protein (RFP), mCherry, and other orange/red fluorophores when compatible fluorescence filter sets are used.

Key features of the kit:

- Enables fast, easy determination of cellular viability with a single assay.
- Suitable for use with fluorescent microscopes (Figure 4), High Content Analysis (HCA) multi-well plate fluorescence scanners (Figure 5), and other fluorescence detection systems.
- Flexible protocol can be adapted for most eukaryotic cell types, including adherent and non-adherent cells.
- Compatible with multiplexing assays.

The LIVE/DEAD™ Viability/Cytotoxicity Assay Kit uses the cell-permeant Calcein, AM to distinguish live cells based on the presence of ubiquitous intracellular esterase activity. In live cells, nonfluorescent calcein is enzymatically converted to fluorescent calcein, which is well-retained within cells and produces an intense uniform green fluorescence (Ex/Em: ~495 nm/~515 nm) (Figure 1). The assay simultaneously detects dead cells, using SYTOX™ Deep Red Nucleic Acid Stain. SYTOX™ Deep Red Nucleic Acid Stain penetrates cells with damaged membranes and shows increased fluorescence with increasing concentrations of dsDNA (Figure 3). Nuclei of dead cells or cells with compromised plasma membranes fluoresce a bright deep red/far red (Ex/Em: maxima 660 nm/682 nm) (Figure 2).

Contents and storage

Catalog numbers that appear as links open the web pages for those products.

Table 1 LIVE/DEAD™ Viability/Cytotoxicity Assay Kit (Cat. No. [L32250](#))

Component	Contents	Concentration	Ex/Em maxima	Amount	Storage
A (green cap)	Calcein, AM (live cell indicator)	2,000X (4 mM) solution in DMSO	494/517 nm	40 µL	<ul style="list-style-type: none"> • ≤ -20°C • Store upright in a dry place • Protect from light
B (purple cap)	SYTOX™ Deep Red Nucleic Acid Stain (dead cell indicator)	2,000X (500 µM) solution in DMSO	660/682 nm	40 µL	

Procedural guidelines

- Reagents in stock solution are susceptible to moisture. Unused solution should be tightly sealed and stored dry at -20°C.
- The protocol provided in this guide is optimized for imaging HeLa, U2OS, A549, BPAE, and HASMC cells, using an EVOS™ M7000 Imaging System, CellInsight™ CX7 High-Content Screening (HCS) Platform, or Countess™ II FL Automated Cell Counter. If needed, the protocol can easily be adapted for other cell types or imaging systems.

Before you begin

Thaw reagents

Remove the reagent stock solutions from the freezer, then allow them to warm to room temperature.

Prepare working solution (1X)

Note: The following procedure is optimized for use with HeLa, U2OS, A549, BPAE, and HASMC cells, but can be adapted for other cell types. SYTOX™ Deep Red Nucleic Acid Stain and Calcein, AM can be used over a range of 0.1 μ M to 1 μ M.

IMPORTANT! Prepare the working solution (1x) on the day of use.

1. Transfer 5 μ L of SYTOX™ Deep Red Nucleic Acid Stain (Component B) to 10 mL of cell culture media or other cell-compatible buffer, then vortex thoroughly. The final concentration of the diluted Component B solution is 0.25 μ M.
2. Transfer 5 μ L of Calcein, AM (Component A) to the diluted Component B solution, then vortex thoroughly. The final concentration of Calcein, AM in solution is 2 μ M.

Protocol

1. Add working solution (1x) to cells, then incubate for 30 minutes at room temperature.
2. (Optional) Wash cells with PBS or other cell-compatible media to optimize the signal-to-noise ratio.
3. Image and count cells to determine cellular viability.
 - For live cells—use a GFP/FITC filter set.
 - For dead cells—use a deep red/Cy5™ filter set.

Spectral properties of Calcein, AM and SYTOX™ dyes

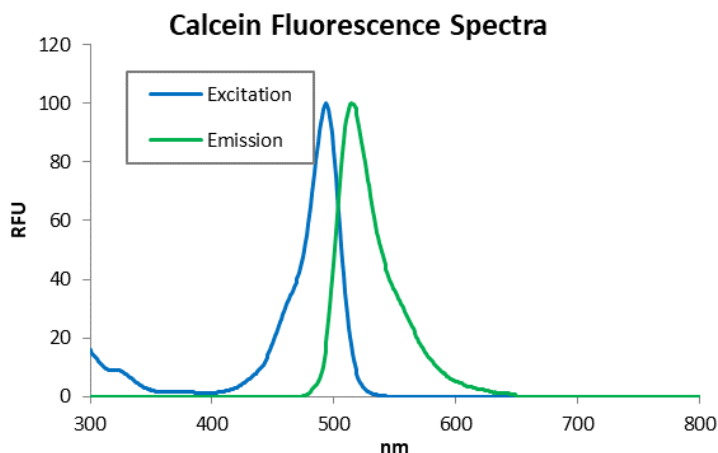


Figure 1 Fluorescence excitation and emission spectra of Calcein, AM.

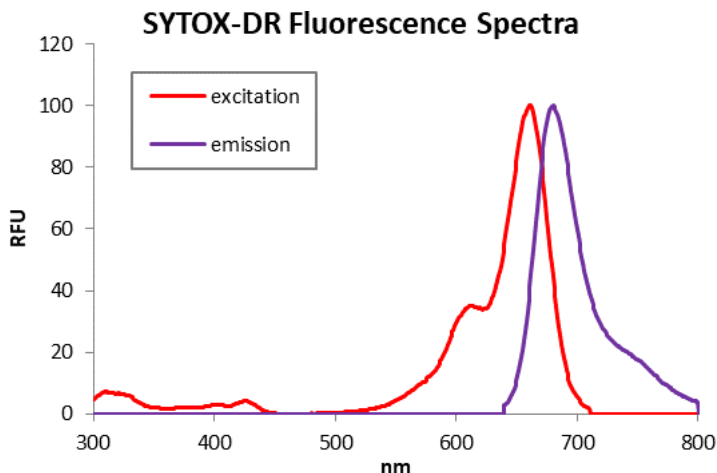


Figure 2 Fluorescence excitation and emission spectra of SYTOX™ Deep Red Nucleic Acid Stain bound to DNA. These spectra were obtained using dsDNA in Tris buffer and a Tecan™ Infinite M1000 plate reader.

Performance characteristics and expected results

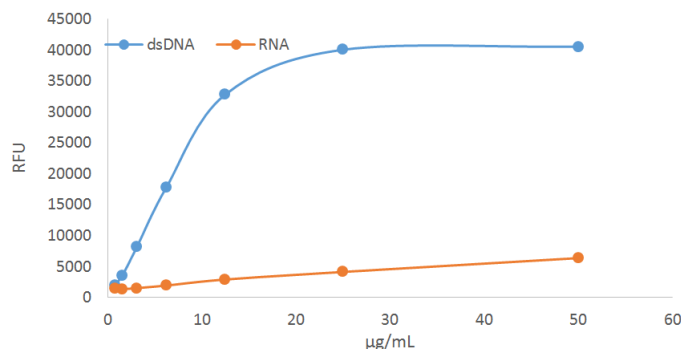


Figure 3 SYTOX™ Deep Red dsDNA vs RNA selectivity.

SYTOX™ Deep Red Nucleic Acid Stain was titrated with varying concentrations of dsDNA or hairpin RNA in Tris buffer using a Tecan™ Infinite M1000 plate reader.

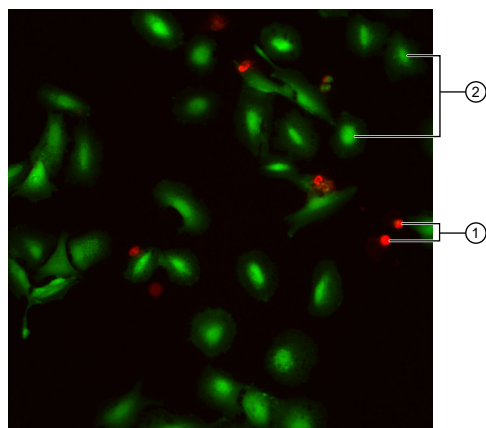


Figure 4 Live/Dead detection on fluorescence microscope. A549 cells were plated in a 96-well plate at a density of 5,000 cells/well in complete media. The cells were grown overnight, then treated with 5 μ M niclosamide for 24 hours. The media was then removed and replaced with Live Cell Imaging Solution (Cat. No. [A14291DJ](#)), containing Calcein, AM and SYTOX™ Deep Red Nucleic Acid Stain from the LIVE/DEAD™ Viability/Cytotoxicity Assay Kit (Cat. No. [L32250](#)).

- ① Dead cells: labeled with SYTOX™ Deep Red Nucleic Acid Stain and shown as red.
- ② Live cells: labeled with Calcein, AM and shown as green.

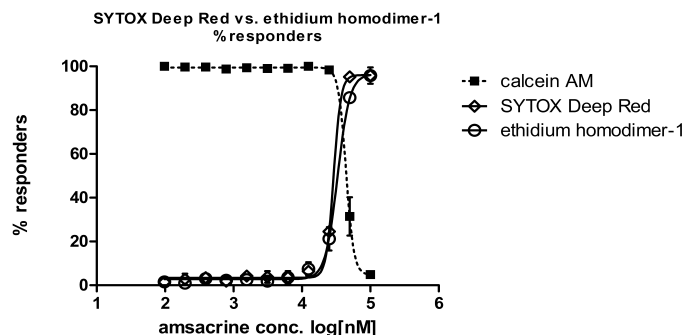


Figure 5 Dose response curve of amsacrine drug while detecting viability of U2OS cells.

U2OS cells were plated in a 96-well plate at a density of 5,000 cells/well in complete media. The cells were grown overnight, then treated with serial 1:2 dilutions of amsacrine, starting at 100 μ M, for 24 hours. The media was then removed and replaced with Live Cell Imaging Solution (Cat. No. [A14291DJ](#)), containing Hoechst 33342 (Cat. No. [H3570](#)), plus either reagents from the LIVE/DEAD™ Viability/Cytotoxicity Assay Kit (Calcein, AM and SYTOX™ Deep Red Nucleic Acid Stain) (Cat. No. [L32250](#)) or the LIVE/DEAD™ Viability/Cytotoxicity Kit, for mammalian cells (Calcein, AM and ethidium homodimer-1 reagents) (Cat. No. [L3224](#)). The plate was incubated for 30 minutes, then analyzed on a CellInsight™ CX5 High Content Screening Platform (HCS). Data was plotted as a nonlinear fit of drug concentration vs. % responders, using GraphPad™ Prism. An apparent $\log IC_{50}$ for amsacrine on U2OS cells is observed when using Calcein, AM (4.647 vs. 4.550) paired with either SYTOX™ Deep Red Nucleic Acid Stain (4.463) or ethidium homodimer-1 (4.521) as the dead cell indicator. This suggests that the two dead cell dyes act equivalently when used in a drug dose response model.

Related products

Table 2 LIVE/DEAD™ kits with Calcein, AM as live cell indicator

Product	Cat. No.	Components	Detection filter set	Ex/Em maxima
LIVE/DEAD™ Viability/Cytotoxicity Kit, for mammalian cells (green/orange)	L3224	Calcein, AM (live cell indicator)	FITC/GFP	494/517 nm
		ethidium homodimer-1 (dead cell indicator)	TRITC/RFP	528/617 nm
LIVE/DEAD™ Cell Imaging Kit (green/red) ^[1]	R37601	Calcein, AM (live cell indicator)	FITC/GFP	494/517 nm
		Dead Red (dead cell indicator)	Texas Red™	570/602 nm

^[1] Available in ReadyProbes™ ready-to-use format.

Limited product warranty

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Revision history: Pub. No. MAN0025668

Revision	Date	Description
A.0	19 August 2021	New document created as part of new product introduction.

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