HCS LIVE/DEAD® Green Kit

Catalog no. H10290

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Image-iT® DEAD Green™ viability stain (Component A)	10 µL	1 mM solution in DMSO	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this kit is stable for 1 year.
Hoechst 33342 (Component B)	25 µL	10 mg/mL aqueous solution		
HCS NuclearMask™ Deep Red stain (Component C)	100 µL	250X concentrate in DMSO		
Number of assays: Sufficient material is supplied for 2 × 96-well plates based on the protocol below.				
Approximate fluorescence excitation/emission maxima: Hoechst 33342: 350/461 nm, bound to DNA; Image-iT® DEAD Green™ viability stain: 488/515 nm, bound to DNA; HCS NuclearMask™ Deep Red stain: 638/686 nm, bound to DNA.				

Introduction

High content imaging and analysis offers the benefit of spatially resolved and multi-parametric interrogation of cells in heterogenous populations. These advantages are particularly evident in studies related to cellular stress and death wherein richer data sets can provide more conclusive results and help to define the next series of questions in characterizing mechanisms of toxicity. The measurement of cell viability represents a fundamental aspect of cytotoxicity.

The HCS LIVE/DEAD® Green Kit is the latest addition to a long line of cell-based assays from Invitrogen to measure cell viability. This kit includes Image-iT® DEAD Green™ viability stain for discrimination of dead cells and HCS NuclearMask™ Deep Red stain or Hoechst 33342 for total cell demarcation. The Image-iT® DEAD Green™ viability stain is impermeant to healthy cells that can gain entrance once the plasma membrane integrity of cells is compromised by drugs, test compounds, or other agents. The Image-iT® DEAD™ Green viability stain is amenable to fixation and permeabilization, and allows for multiplexing with other biomarkers of cytotoxicity.

As an example, the HCS LIVE/DEAD® Green Kit was used to detect and quantitate plasma membrane permeability in HeLa cells treated with valinomycin (Figures 1 and 2) and validated for robustness and signal change (Tables 2 and 3).

The HCS LIVE/DEAD® Green Kit contains sufficient material to perform the viability assay on two 96-well plates when used as described in the protocol below. For larger quantities, inquire at www.invitrogen.com.

Materials Required but Not Provided

- Flat-bottom 96-well plates
- Phosphate buffered saline (PBS, Invitrogen Cat. no. 14190-144)
- Complete medium (e.g., for HeLa cells, Dulbecco's Modified Eagle Medium, Invitrogen Cat. no. 11095-114)
- Paraformaldehyde 16% aqueous solution

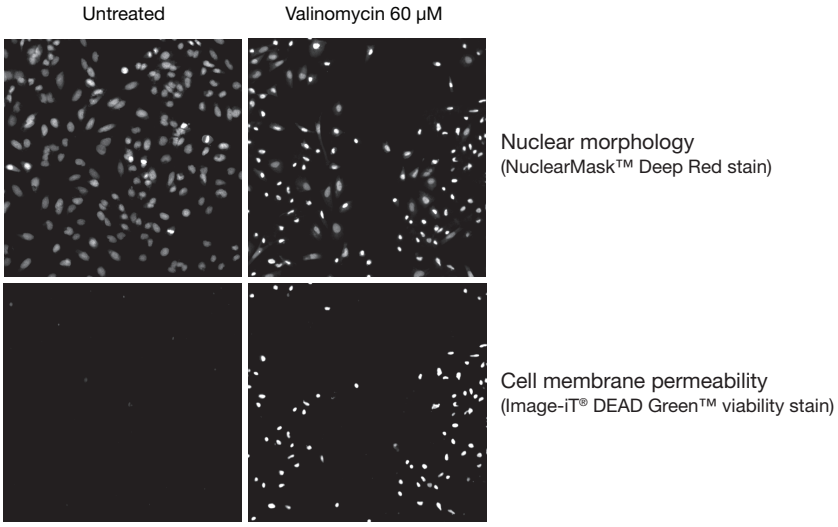

Figure 1. HeLa cells were treated with 60 µM of valinomycin for 24 hours at 37°C and toxicity was assayed using the Image-iT® DEAD Green™ viability stain and HCS NuclearMask™ Deep Red stain from the HCS LIVE/DEAD® Green Kit. Imaging and analysis was performed using a 10X objective and the Compartmental Analysis BioApplication with the Thermo Scientific Cellomics® ArrayScan® VTI platform. Nuclear segmentation was performed using HCS NuclearMask™ Deep Red stain.

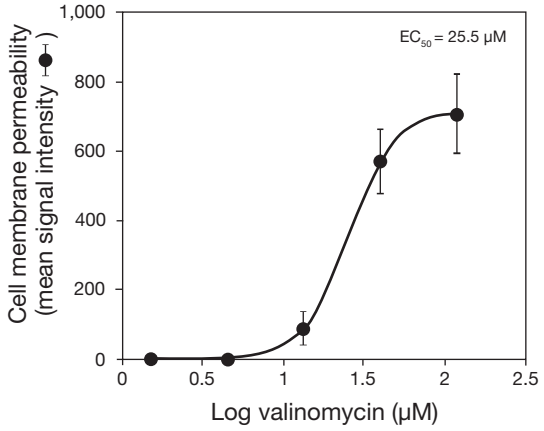

Figure 2. Dose response for valinomycin in HeLa cells using the HCS LIVE/DEAD® Green Kit. HeLa cells were treated with valinomycin at final concentrations between 0 to 120 µM and incubated for 24 hours at 37°C/5% CO_2. Dose response curves generated by non-linear regression with GraphPad PRISM® were used to determine EC_{50} values for valinomycin-induced loss of plasma membrane integrity with increasing concentrations of valinomycin. The EC_{50} value was calculated using mean signal intensity values of Image-iT® DEAD Green™ viability stain in the nucleus plotted against various doses of valinomycin. Data points represent averages from 8 wells and the error bars show standard deviations. **Note:** Since valinomycin is less soluble in DMSO at concentrations above 120 µM, data points beyond this concentration are not shown in the figure.

Table 2. Assay robustness.*

Analyzed parameter	CV of treated samples (%)
Cell membrane permeability	16 ± 2.1

*HeLa cells were treated with 120 µM valinomycin for 24 hours at 37°C/5% CO_2 and cytotoxicity was assayed using the HCS LIVE/DEAD® Green Kit. Quantitative analysis was performed using the Thermo Scientific Cellomics® ArrayScan® VTI and Compartmental Analysis Bioapplication. The data represents % CVs of the averages and standard deviations from treated samples (Max) of three Min/Max plates. CV values were <20% for this parameter.

Table 3. Quantitation of cytotoxicity.*

Analyzed parameter	Signal change by treatment (-fold)
Cell membrane permeability	190 ± 41

*HeLa cells were treated with 120 µM valinomycin for 24 hours at 37°C/5% CO_2 and cytotoxicity was assayed with the HCS LIVE/DEAD® Green Kit. Quantitative analysis was performed using the Thermo Scientific Cellomics® ArrayScan® VTI and Compartmental Analysis BioApplication. The average intensities and standard deviations were calculated for cell membrane permeability. The data shown represents the average fold change in signal intensities of treated samples (Max) from three Min/Max plates. The -fold change in signal between treated and control samples was >3-fold indicating cytotoxicity.

Caution

- Hoechst 33342 (Component B) is a known mutagen. Use the dye with appropriate precautions.
- DMSO (in Components A and C), is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations. Always wear protective laboratory clothing and gloves when handling this reagent.

Preparing Cells

Plate cells in appropriate medium the day before adding the test compound. For adherent cells, optimize the cell number and plate coating requirements for the chosen cell model and time span of test compound treatment before performing assay.

Preparing Stock Solutions

Determine whether you will use the blue fluorescent Hoechst 33342 (Component B) or the infrared fluorescent HCS NuclearMask™ Deep Red stain (Component C) as the live cells stain with the green fluorescent Image-iT® DEAD Green™ viability stain. Only one live cell stain is required to perform the assay.

Prepare the following solutions **fresh** on the day of the assay. The following protocol prepares sufficient material to stain one 96-well plate.

Open DMSO containing stock solutions only after the contents are equilibrated to room temperature.

1.1 On the day of the assay, prepare the cell staining solution as follows:

- If using Hoechst 33342, add 2.1 µL Image-iT® DEAD Green™ viability stain (Component A) to 6 mL complete medium. Hoechst 33342 is prepared in step 1.2.
- If using HCS NuclearMask™ Deep Red stain, add 40 µL of HCS NuclearMask™ Deep Red stain and 2.1 µL Image-iT® DEAD Green™ viability stain to 6 mL complete medium.

1.2 Prepare the fixation solution by adding 3 mL of 16% paraformaldehyde to 9 mL PBS .

- If using Hoechst 33342, add 6 µL of Hoechst 33342 (Component B) directly to 3 mL of 4% fixation solution.

Experimental Protocol

Labeling Cells in 96-well Plates for Imaging

This labeling protocol was developed using HeLa cells. For other cell types, you may need to modify the protocol appropriately. See Figure 3 for HCS LIVE/DEAD® Green Kit workflow.

2.1 Add test compound or drug to cells to a total volume of 125 µL and incubate for the desired period of time under normal cell culture conditions.

Note: DMSO concentrations should not be higher than 0.5% in the incubation with live cells. When adding drugs or test compounds at this step, consider the additional 0.06% DMSO introduced with the Image-iT® DEAD Green™ viability stain. If other organic solvents are used for preparing drug stock solutions, determine their compatibility with the cells.

2.2 Do not remove the incubation medium from wells of the 96-well plate after the test compound or drug treatment.

2.3 Add 50 µL of the cell staining solution (prepared in step 1.1) to each well for a total volume of 175 µL and incubate the plate under normal cell culture conditions for 30 minutes.

2.4 Remove medium.

2.5 Add 100 µL of fixation solution (prepared in step 1.2) to each well and incubate for 15 minutes at room temperature.

2.6 Remove the fixation solution and wash wells once with PBS.

2.7 Add 100 µL of PBS to each well and proceed to **Imaging and Analysis**.

Imaging and Analysis

Scan the plate using an automated imaging platform equipped with filters appropriate for Hoechst and FITC or Cy®5 and FITC (if using HCS NuclearMask™ Deep Red stain). The nucleus is characterized by Hoechst 33342 in the Hoechst channel or HCS NuclearMask™ Deep Red stain in the Cy®5 channel. Cell membrane permeability is assessed by determining signal intensity increase in the nuclear regions in the FITC channel.

When using the Thermo Scientific Cellomics® ArrayScan® VTI platform, use the Compartmental Analysis BioApplication. In channel 1, define the nucleus with Hoechst 33342 or HCS NuclearMask™ Deep Red stain (the segmentation tool) as objects with Hoechst/XF93 or Cy®5 dye/XF93, filters, respectively. In channel 2, assess the nuclear fluorescence intensity of Image-iT® DEAD Green™ viability stain with FITC/XF93 filters.

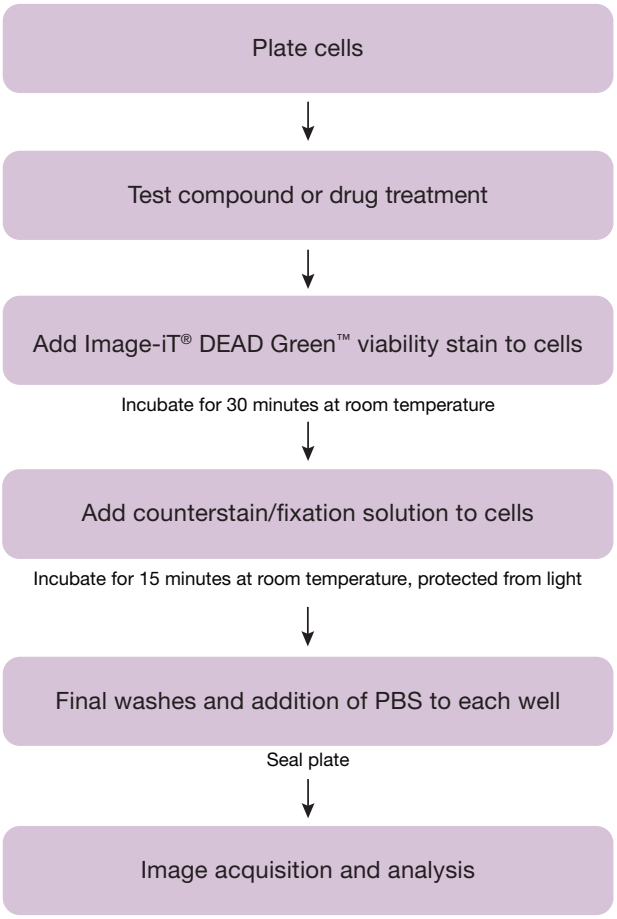

Figure 3. Workflow for the HCS LIVE/DEAD® Green Kit.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
H10290	HCS LIVE/DEAD® Green Kit *2-plate size*	1 kit
Related Products		
C10289	Click-iT® AHA Alexa Fluor® 488 Protein Synthesis HCS Assay *2-plate size*	1 kit
C10045	CellMask™ Orange plasma membrane stain *5 mg/mL solution in DMSO*	100 µL
C10046	CellMask™ Deep Red plasma membrane stain *5 mg/mL solution in DMSO*	100 µL
H10292	HCS DNA Damage Kit *2-plate size*	1 kit
H10294	HCS NuclearMask™ Deep Red stain *250X concentrate in DMSO*	400 µL
H10293	HCS Mitotic Index Kit *2-plate size*	1 kit
H10295	HCS Mitochondrial Health Kit *2-plate size*	1 kit
H32711	HCS CellMask™ Red cytoplasmic/nuclear stain *5 mM solution in DMSO* *for high content screening* *for cellular imaging*	125 µL
H34558	HCS CellMask™ Blue cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set
H34560	HCS CellMask™ Deep Red cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set
H34157	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *2-plate size*	1 kit
H34158	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *10-plate size*	1 kit
H34350	HCS LipidTOX™ Green phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* 10-plate size*	each
H34351	HCS LipidTOX™ Red phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* 10-plate size*	each
H34475	HCS LipidTOX™ Green neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34476	HCS LipidTOX™ Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34477	HCS LipidTOX™ Deep Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
I10291	Image-iT® DEAD Green™ viability stain *1 mM solution in DMSO*	25 µL

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