HCS Mitochondrial Health Kit

Catalog no. H10295

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.
MitoHealth stain (Component B)	each	solid		
Hoechst 33342 (Component C)	25 µL	10 mg/ml in water		
Dimethylsulfoxide (DMSO, Component D)	100 µL	Not applicable		
*These storage conditions are for storing the kit upon receipt. For optimal storage of each component, see vial labels.				
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; MitoHealth stain: 550/580 nm.				

Introduction

The mitochondrial membrane potential is a central feature of healthy mitochondria. It is essential in Ca^{2+} uptake and storage, reactive oxygen species generation and detoxification and, most importantly, the synthesis of ATP by oxidative phosphorylation.[1] Therefore, its depolarization is a good indicator of mitochondrial dysfunction, which is increasingly implicated in drug toxicity.[2–7]

The HCS Mitochondrial Health Kit was developed for simultaneous quantitative measurements of two cell health parameters by high content analysis in the same cell: mitotoxicity and cytotoxicity. The MitoHealth stain accumulates in mitochondria in live cells proportional to the mitochondrial membrane potential (Figure 1, bottom panels). Cytotoxicity is measured with the Image-iT® DEAD Green™ viability stain. The Image-iT® DEAD Green™ viability stain has a high affinity for DNA and forms highly fluorescent and stable dye-nucleic acid complexes; it is non-fluorescent when not bound to DNA. Staining of nuclear DNA can not occur in live cells due to the impermeability of the cell membrane for the stain. Drugs and test compounds leading to serious cell injuries, including cell membrane permeability, allow entry of the stain. This property enables the discrimination of dead cells with Image-iT® DEAD Green™ viability stain (Figure 1, center panels). Hoechst 33342, which stains nuclear DNA in live and dead cells, is supplied in this kit as a segmentation tool for automated image analysis (Figure 1, top panels). All dyes included in this kit have sufficient retention of fluorescence signal intensity upon formaldehyde fixation and detergent permeabilization to be useful in fixed endpoint assays, as well as applications involving immunocytochemistry. Valinomycin treatment of HeLa cells is shown as an example for assay validation including robustness (Table 2), signal intensity change upon treatment (Table 3) and generation of EC_{50} values (Figure 2). The assay was performed using the Thermo Scientific Cellomics® ArrayScan® VTI platform, employing the Compartmental Analysis

BioApplication to measure changes in fluorescence intensity in the nuclear regions and in spots within the rings around nuclear regions.

This kit contains sufficient material to perform the HCS Mitochondrial Health assay on two 96-well plates when used as described in the protocol. For larger quantities, inquire at www.invitrogen.com.

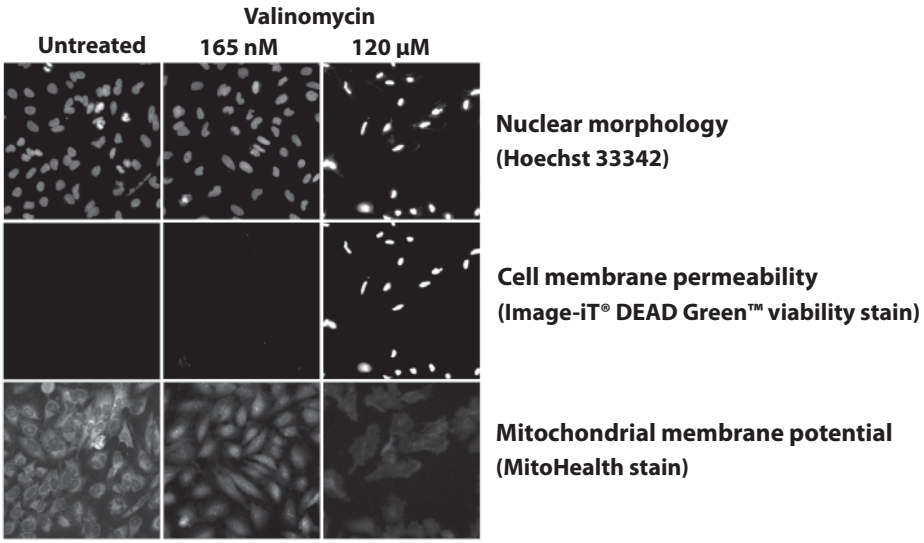

Figure 1. Imaging of mitotoxicity and cytotoxicity of valinomycin in HeLa cells using the HCS Mitochondrial Health Kit. HeLa cells were treated in a dose response experiment with valinomycin between 2 nM and 120 µM final concentration or with an equal volume of solvent only (DMSO, untreated), incubated for 24 hours at 37°C/5% CO_2, and assayed using the HCS Mitochondrial Health assay. The Thermo Scientific Cellomics® ArrayScan® VTI platform was used to obtain images of fixed cells at 20x. In the absence of valinomycin, the nucleus (detected with Hoechst 33342) and mitochondrial reticulum (detected with MitoHealth stain) were clearly visible, yet no signal due to permeability of plasma membranes (Image-iT® DEAD Green™ viability stain) was observed. At valinomycin concentrations in the nanomolar range, the mitochondrial reticulum was not observed as a defined structure, indicating valinomycin-induced loss of mitochondrial membrane potential. Loss of plasma membrane integrity did not occur at this level of valinomycin exposure, hence there was no fluorescence staining by the Image-iT® DEAD Green™ viability stain. At higher valinomycin concentrations (120 µM), however, plasma membrane integrity was compromised and led to intense nuclear fluorescence from the Image-iT® DEAD Green™ viability stain, indicating more overt cytotoxicity and cell death.

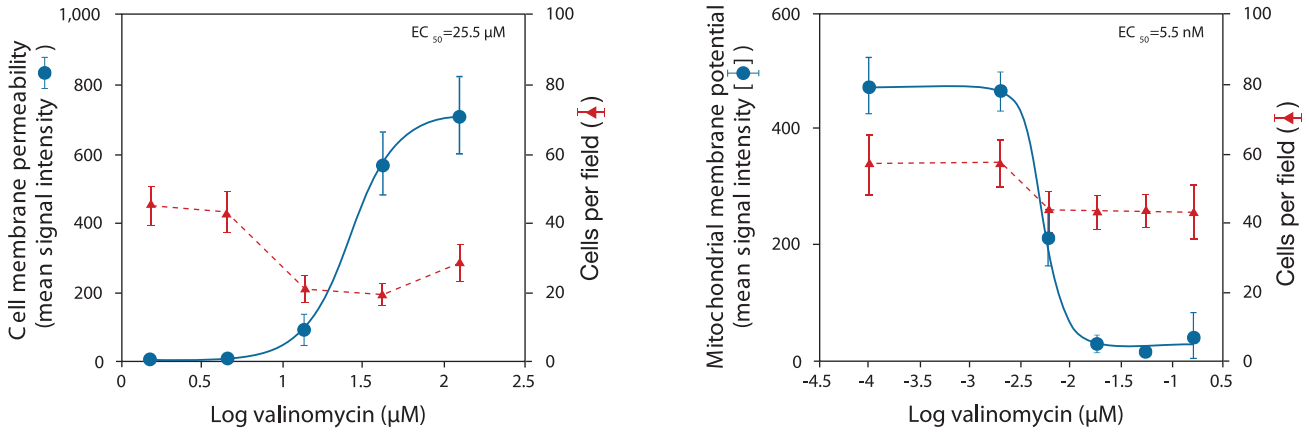

Figure 2. Dose response for valinomycin in HeLa cells using the HCS Mitochondrial Health Kit. HeLa cells were treated with valinomycin at final concentrations between 2 nM and 120 µM. Cells were incubated for 24 hours at 37°C/5% CO_2 and assayed using the HCS Mitochondrial Health Kit. The Thermo Scientific Cellomics® ArrayScan® VTI platform with Compartmental Analysis BioApplication was used for analysis. 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 (left) and mitochondrial membrane potential (right) with increasing concentrations of valinomycin. Cell loss with increasing drug concentrations is shown in both panels. 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	16.0% ± 2.1%
Mitochondrial membrane potential	14.6% ± 2.8%

*HeLa cells were treated with 120 µM valinomycin in DMSO (treated) or an equal amount of DMSO solvent only (untreated), incubated for 24 hours at 37°C/5% CO_2 and assayed using the HCS Mitochondrial Health Kit. Quantitative analysis was performed using the Thermo Scientific Cellomics® ArrayScan® VTI and Compartmental Analysis Bioapplication. The average and standard deviation were calculated of the CVs obtained from treated samples (Max) for 3 Min/Max plates from 48 treated samples. CV values were <20% for both parameters.

Table 3. Quantitation of mitotoxicity and cytotoxicity.*

Analyzed parameter	Signal change by treatment (-fold)
Cell membrane permeability	190 ± 41
Mitochondrial membrane potential	3.2 ± 0.3

*HeLa cells were treated with 120 µM valinomycin in DMSO (treated) or an equal amount of DMSO solvent only (untreated), incubated for 24 hours at 37°C/5% CO_2 and assayed using the HCS Mitochondrial Health 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 each output parameter, cell membrane permeability which measures cytotoxicity and mitochondrial membrane potential. The average and standard deviation were calculated of the ratios of signal intensities of 48 treated (Max) vs 48 untreated (Min) samples obtained from 3 Min/Max plates. The fold change in signal between treated samples and control was >3-fold for both parameters indicating mitotoxicity and cytotoxicity.

Before You Begin

Materials Required but Not Provided

- 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 (Polysciences Cat. no. 18814)

Caution

- Hoechst 33342 (Component C) is a known mutagen. Use the dye with appropriate precautions.
- DMSO (in Components A and D), 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. For analyzing labeled mitochondria, avoid sparsely or overly confluent cells. For example, HeLa and A549 cells were plated at 5,000 cells per well and allowed to grow to 80% confluency before treatment with valinomycin.

Preparing Stock Solutions

Prepare the cell staining solution and counterstain/fixation solution **fresh** on the day of the assay. The following protocol prepares sufficient material to stain one 96-well plate.

1.1 Prepare MitoHealth stain solution by dissolving the MitoHealth stain (Component B) in 30 µL DMSO (Component D). Store any unused solution at –20°C, desiccated and **protected from light**.

1.2 **On the day of the assay**, prepare the cell staining solution by adding 10.5 µL of the MitoHealth stain solution (prepared in step 1.1) and 2.1 µL of Image-iT® DEAD Green™ viability stain (Component A) to 6 mL complete medium.

Note: DMSO is hygroscopic. Open DMSO containing stock solutions only after the vial is equilibrated to room temperature.

1.3 Prepare the counterstain/fixation solution by adding 3 mL 16% paraformaldehyde and 6 µL of Hoechst 33342 (Component C) to 9 mL PBS.

Experimental Protocol

Labeling Cells in 96-well Plates for Imaging

This protocol was developed using HeLa and A549 cells. For other cell types, you may need

to modify the protocol. See Figure 3 for HCS Mitochondrial Health Kit workflow.

2.1 Add test compound or drug to cells and incubate for the desired period of time under normal cell culture conditions. The total volume in each well should be 125 µL at this point.

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 cell staining solution. If other organic solvents are used for preparing drug stock solutions, determine their compatibility with cells.

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

2.3 Add 50 µL of the cell staining solution (prepared in step 1.2) to each well and incubate the plate under normal cell culture conditions for 30 minutes. The total volume in each well should be 175 µL at this point.

2.4 Remove medium.

2.5 Add 100 µL of counterstain/fixation solution (prepared in step 1.3) to each well and incubate for 15 minutes at room temperature.

2.6 Remove counterstain/fixation solution and wash wells once with 100 µL PBS.

2.7 Add 200 µL 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 DAPI/Hoechst, FITC, and TRITC. The nucleus is characterized by Hoechst 33342 in the DAPI/Hoechst channel. Cell membrane permeability is assessed by determining signal intensity increase in the nucleus in the FITC channel. Mitochondrial toxicity is measured by the signal decrease in the TRITC channel in spots within a ring around the nucleus.

When using the Thermo Scientific Cellomics® ArrayScan® VTI platform, use the Compartmental Analysis BioApplication. In channel 1, define the nucleus with Hoechst 33342 (the segmentation tool) as objects with Hoechst/XF93 filters. In channel 2, assess the nuclear fluorescence intensity of Image-iT® DEAD Green™ viability stain with FITC/XF93 filters. In channel 3, determine the fluorescence intensity of MitoHealth stain using TRITC/XF93 filters in spots within the rings around the nucleus outlined in channel 1.

References

1. Aging Cell 3, 35 (2004); **2.** Toxicol Sci.69, 131 (2002); **3.** Curr Med Chem 12, 1829 (2005); **4.** Arch Toxicol 80, 580 (2006); **5.** Drug Discovery Today 12, 777 (2007); **6.** Toxicol Sci 103, 335 (2008); **7.** J Biomol Screen 13, 527 (2008).

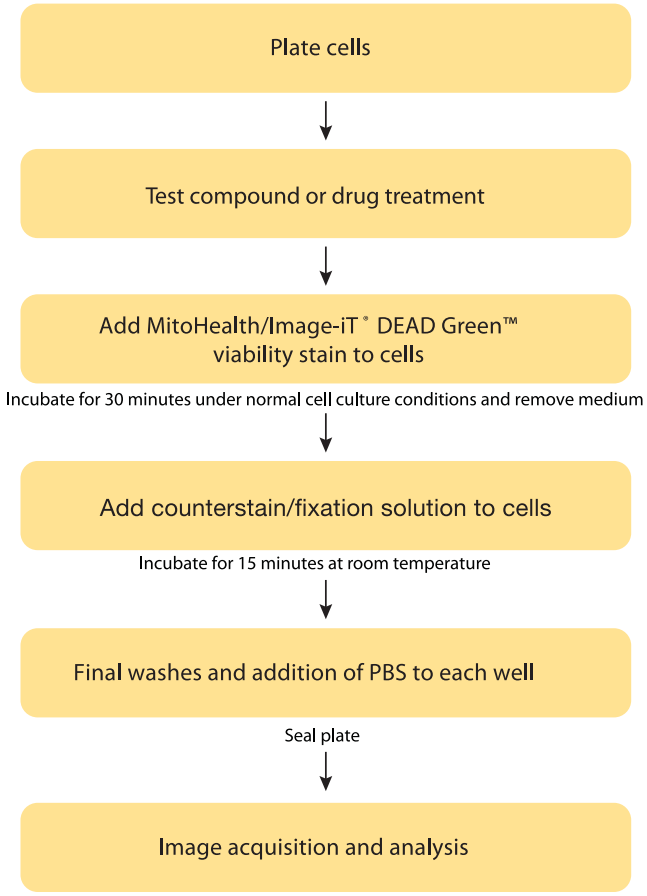

Figure 3. Work Flow for the HCS Mitochondrial Health Kit.

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

Cat. no.	Product Name	Unit Size
H10295	HCS Mitochondrial Health 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
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* *2-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*	each

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
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Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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