Image-iT® DEAD Green™ Viability Stain

Catalog no. I10291

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Image-iT® DEAD Green™ viability stain	25 µL	1 mM solution in DMSO	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this product is stable for 6 months.
Number of assays: Sufficient reagents are supplied for 25 × 96-well plates based on assay volumes of 100 µL per well or 250 coverslips based upon a 1 mL volume.				
Approximate fluorescence excitation/emission maxima: Image-iT® DEAD Green™ viability stain: 488/515 nm.				

Introduction

Cytotoxicity is a multi-parametric process resulting in plasma membrane permeability. Image-iT® DEAD Green™ viability stain is an impermeant dye to healthy cells that becomes permeant when the plasma membrane integrity of cells is compromised. In contrast to viability stains like propidium iodide, Image-iT® DEAD Green™ viability stain is amenable to fixation and permeabilization, and allows for multiplexing with other cytotoxicity biomarkers. Image-iT® DEAD Green™ viability stain can be used in high content screening (HCS) assays and standard fluorescent microscopy imaging applications (Figure 1).

Sufficient material of Image-iT® DEAD Green™ viability stain is supplied to assay 25 plates in a 96-well plate format and 100 µL well volume or 250 coverslips based upon a 1 mL volume.

Before Starting

Materials Required but Not Provided

- Phosphate buffered saline (PBS, Invitrogen Cat. no. 14190-144)
- Complete medium suitable for the cell type used
- Paraformaldehyde 16% aqueous solution (e.g., Polysciences Cat. no. 18814)
- Flat-bottom 96-well microplates or coverslips
- *Optional:* Triton® X-100, blocking solution, primary antibody, and secondary antibody (required if staining with antibodies)

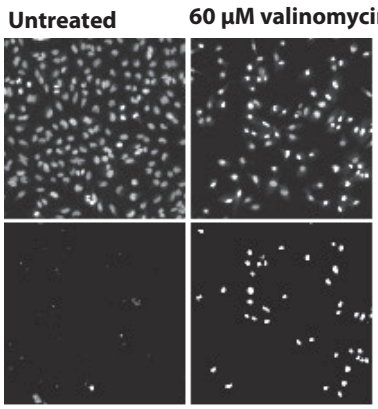

Figure 1. HeLa cells were treated with DMSO (untreated) or 60 µM valinomycin for 18 hours, stained with Image-iT® DEAD Green™ viability stain for 30 minutes, fixed, and imaged on Thermo Scientific Cellomics® ArrayScan® VTI.

Caution DMSO in the stain 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. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective 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 Solutions Prepare the fixative and permeabilization solution **fresh** on the day of the assay. The following recipe is to prepare sufficient solutions to stain 1 × 96-well plate or 10 coverslips.

1.1 Prepare the fixative solution by adding 1.5 mL 16% aqueous paraformaldehyde solution to 4.5 mL PBS to obtain a 4% paraformaldehyde solution.

1.2 Prepare the permeabilization solution by adding 7.5 µL of Triton® X-100 to 7.5 mL PBS to obtain a 0.1% Triton® X-100 solution. This solution is required if you are performing staining with antibodies.

Experimental Protocols

See Figure 2 for the Image-iT® DEAD Green™ viability stain workflow.

The Image-iT® DEAD Green™ viability stain works well with fixation and permeabilization and can be successfully used for multiplexing with other markers. If sample permeabilization is necessary in HCS applications, use Triton® X-100 at a concentration of 0.1% for 10 minutes and image samples within 24 hours after staining. If performing traditional fluorescence microscopy at higher magnifications, detergent permeabilization of sample may result in an increase of cytosolic background. When discriminating live from dead cells, compare differences in the nuclear regions.

Detecting Viability with Image-iT® DEAD Green™ Viability Stain

2.1 Treat cells with a test compound or drug and incubate for the recommended time.

Note: Do not remove the incubation medium after test compound or drug treatment.

2.2 Add Image-iT® DEAD Green™ viability stain at a final concentration of 100 nM to the cells and incubate for 30 minutes at 37°C.

2.3 Remove medium.

2.4 Add fixative solution prepared in step 1.1 to each well or coverslip as indicated below and incubate for 15 minutes at room temperature:

- If using microplates, add 100 µL to each well.
- If using coverslips, add 1.0 mL to each coverslip.

2.5 Remove fixative solution and rinse cells three times with PBS.

Proceed to **Antibody Detection**, if desired. If no additional staining is needed, proceed to **Imaging and Analysis**.

Antibody Detection (Optional)

3.1 Incubate cells with permeabilization solution prepared in step 1.2 as indicated below for 15 minutes at room temperature:

- If using microplates, add 100 µL to each well.
- If using coverslips, add 500 µL to each coverslip.

3.2 Remove permeabilization solution, then rinse cells three times in PBS.

3.3 Add blocking solution and incubate for the recommended time. Remove blocking solution.

3.4 Prepare primary antibodies (as recommended by the manufacturer).

3.5 Add the primary antibody solution prepared above and incubate for the recommended time.

3.6 Remove the primary antibody solution and wash each well/coverslip. Remove the wash solution.

3.7 Prepare the secondary antibody solution as recommended by the manufacturer.

3.8 Add the secondary antibody solution prepared above and incubate for the recommended time.

3.9 Remove the secondary antibody solution and wash each well/coverslip. Remove the wash solution.

Imaging and Analysis

- If using an automated imaging platform, add PBS to each well and scan plate using appropriate automated imaging platform with filters appropriate for FITC and any other fluorophores utilized. Cell membrane permeability is assessed by determining signal intensity increase in the nucleus in the FITC channel.
- If performing standard fluorescence microscopy, the Image-iT® DEAD Green™ stained cells maybe mounted using suitable buffer for live cells or prepare a mounting medium appropriate for fixed cells. Use approximate fluorescence excitation/emission maxima appropriate for imaging fluorescein or Alexa Fluor® 488 dye.

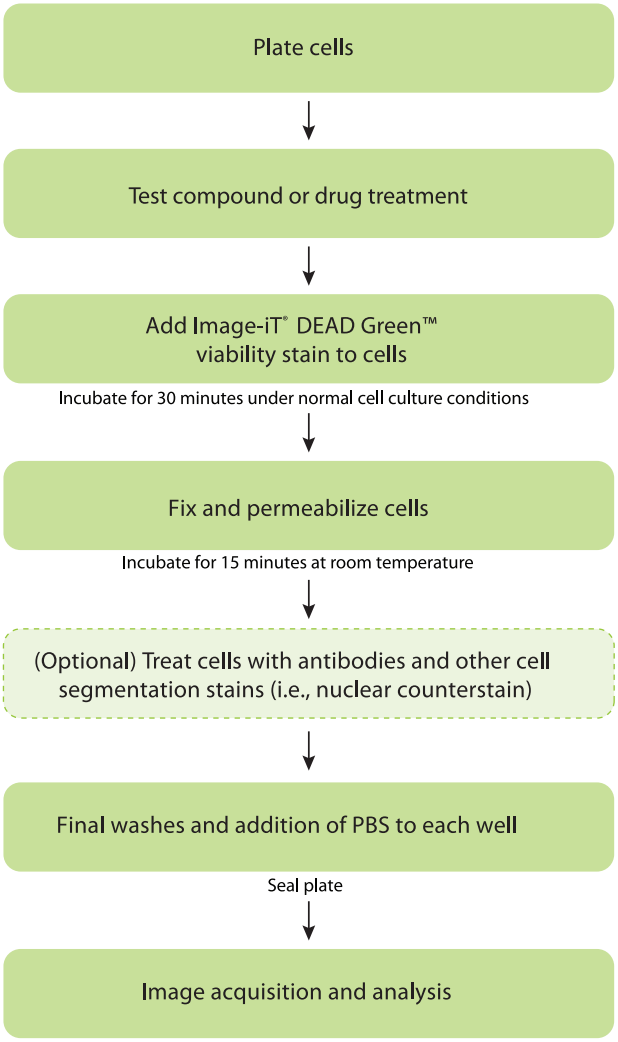

Figure 2. Work flow for Image-iT® DEAD Green™ viability stain.

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

Cat. no.	Product Name	Unit Size
I10291	Image-iT® DEAD Green™ viability stain *1 mM solution in DMSO*	each
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
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* *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

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:
Order Phone: (800) 438-2209
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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

For country-specific contact information, visit www.invitrogen.com.

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