

INSTRUCTIONS

Cellomics[®] Cell Viability Kit

High-Content Screening Reagents

1820.1

Number	Description
K02-0001-1	Cell Viability Kit, sufficient materials for 5 × 96 wells
R02-0002-1	Cell Viability Kit, sufficient materials for 50 × 96 wells

Kit Contents:	K0200011	R0200021
VitalDye	5 x 115 µg	2 x 3.2 mg
DeadDye	1.1 ml	2 x 7.5 ml
Hoechst Dye	2 x 28 µl	745 µl
Wash Buffer	100 ml	--
Thin Plate Seal Assembly	7/pack	--

Storage: Upon receipt store all kit components at 4°C. Keep vials containing the VitalDye, DeadDye and Hoechst Dye solutions protected from light. Allow buffers to warm to room temperature before use. See the **Solution Preparation** section for storage and stability of prepared solutions.

Warning: Please completely read these instructions and the accompanying material safety data sheets before using this product. The Cellomics Reagents are not for diagnostic use in humans or animals.

Introduction

The Thermo Scientific Cellomics Cell Viability Reagent Kit for high-content screening enables quantification of live and dead cells, and total cell counts. The kit provides a fixed end-point assay based on fluorescence detection of live and dead cells distributed on microplates. The core reagents supplied include DeadDye and VitalDye. The nuclei are identified by DNA-specific Hoechst Dye, which is also included in the kit.

When cells are dead or dying, plasma membranes leak and become permeable to large macromolecules. In this state, DeadDye enters the cell and is visualized by its red fluorescence emission. VitalDye, a membrane-permeable fluorescent probe, operates as a live-cell indicator. Soluble cytoplasmic enzymes cleave VitalDye, trapping its fluorescent moiety inside intact cells. Live cells stained with VitalDye are distinguished from dead cells by their green fluorescence emission. Identification of the entire cell population is achieved using Hoechst Dye, a membrane-permeable nucleic acid stain emitting in the blue fluorescence channel. DeadDye and VitalDye may be used separately or in combination to quantify dead cells, live cells or both cell populations (Figure 1).

The Cell Viability Kit Reagents, in combination with the ArrayScan HCS System and the Cell Viability Application software enable automated plate handling, focusing, cell image acquisition, and analysis and cell viability quantification. For a more detailed description of the image processing algorithm, see the Cell Viability Application Guide that accompanies the software.

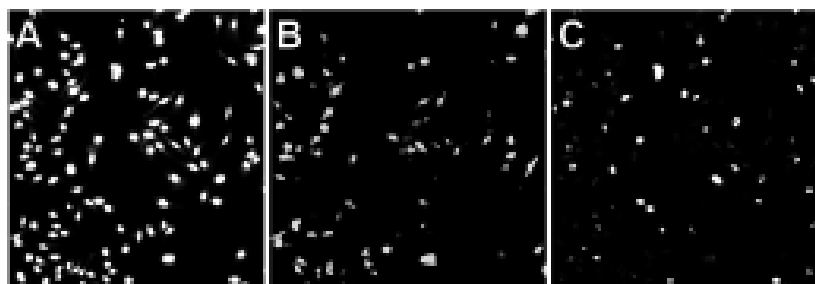


Figure 1. Live and dead cells distinguished by the Cell Viability Kit and the ArrayScan Reader. HeLa cells were prepared as described in the protocol. (A): Hoechst staining identifies all cells in the imaged field. VitalDye (B) and DeadDye (C) show live and dead cells, respectively.

Additional Materials Required

Note: For the screening size kit, Wash Buffer and Permeabilization Buffer are available separately. Call customer service for more information.

- Formaldehyde (37%) (Sigma, Product No. F1268)
- Black, clear bottom microplates (Packard ViewPlate[®], Product No. 6005182)
- Tween-20 Detergent (Thermo Scientific Surfact-Amps[®] 20, Product No. 28320)
- Poly-L-lysine, 30,000-70,000 MW (ICN Biochemicals, Product No. 194543)

Cell Preparation Information

- The protocol is optimized for HeLa cells (ATCC, Product No. CCL-2)
- Culture cells in Minimum Essential Medium-Eagle (EMEM; BioWhittaker, Product No. 12-611Q) containing the following supplements (=EMEM Complete Medium with 10% FBS): 10% fetal bovine serum (BioWhittaker, Product No. 14-503F), 1% L-glutamine (BioWhittaker, Product No. 17-605E), 1% non-essential amino acids (BioWhittaker, Product No. 13-114E), 1% sodium pyruvate (BioWhittaker, Product No. 13-115E), 1% penicillin/streptomycin (BioWhittaker, Product No. 17-602E)
- EMEM with 2% FBS and 2% HEPES: 2% fetal bovine serum, 2% HEPES (Invitrogen, Product No. 15630-080), 1% L-glutamine, 1% non-essential amino acids, 1% sodium pyruvate, 1% penicillin/streptomycin
- 2% HEPES without FBS: 2% HEPES, 1% L-glutamine, 1% non-essential amino acids, 1% sodium pyruvate, 1% penicillin/streptomycin solution
- Split cells when they reach 70-80% confluency (2-3 times per week) at a dilution of 1:2 to 1:6.
- Harvest cells with trypsin-versene mixture (BioWhittaker, cat. # 17-161F), dilute in EMEM complete medium, and determine cell density.
- Dilute cells to 3.75×10^4 cells/ml in EMEM Complete Medium and add 200 μ l of the cell suspension per well of a 96-well microplate (= 7,500 cells/well). Incubate for 18-24 hours at 37°C in 5% CO₂.

Poly-L-lysine-Coated Microplates

1. Add 5 mg of poly-L-lysine to 50 ml sterile, tissue-culture grade water, which is enough working stock to coat 10 plates.
2. Add 40 μ l of working stock to each well and incubate at room temperature for 5 minutes.
3. Remove solution by inverting and shaking plate.
4. Add 100 μ l of sterile water to each well. Remove the water as completely as possible.
5. Allow plate to air-dry in sterile hood for at least 2 hours. Poly-L-lysine-coated plates are stable for 12 months, stored at 4°C.

Cell Viability Kit Protocol

A. Solution Preparation (per 96-well plate)

1X Wash Buffer	Add 20 ml 10X Wash Buffer to 180 ml ultrapure water for a final volume of 200 ml. Store buffer at 4°C for up to 7 days.
VitalDye/DeadDye Solution	Resuspend the VitalDye vial with 25 µl DMSO. Adjust to 24.8 ml final volume in cell culture medium without FBS. Add 200 µl of DeadDye. Prepare just before each assay and warm to 37°C before use. For using DeadDye alone, dilute 200 µl of DeadDye with 24.8 ml cell culture medium without FBS. For using VitalDye alone, resuspend VitalDye vial with 25 µl DMSO and adjust to 25 ml with cell culture medium without FBS.
Fixation/Hoechst Solution	Add 2.5 ml of 37% formaldehyde and 12.3 µl of Hoechst Dye to 22.5 ml of 1X Wash Buffer. Prepare just before each assay and warm to 37°C before use.
Tween-20 Solution	For 8 wells (200 µl/well), dilute 80 µl of Tween-20 Detergent with 2 ml cell culture medium with 2% FBS. Prepare just before each assay and warm to 37°C before use.

B. Procedure

Note: Use 200 µl per well volume unless indicated otherwise. This protocol requires ~2 hours to complete once compound incubation has been completed.

1. Prepare Poly-L-lysine coated microplates.
2. Plate cells in a 96-well plate sufficiently in advance of experiment such that cells are well-attached. Plate 7.5×10^3 HeLa cells/well in 200 µl volume 24 hours before experiment.

Note: Pre-warm cell culture medium with 2% FBS, cell culture medium without FBS, diluted Tween-20, diluted dye solutions, and Fixation/Hoechst Solution to 37°C.

3. Aspirate culture medium and add 200 µl of test compound. For live cell controls, add 200 µl compound diluent to appropriate wells. For dead cell controls, add 200 µl Tween-20 Solution to the appropriate wells.
4. Incubate for 30 minutes at 37°C with 5% CO₂.
5. Aspirate culture medium and wash briefly with pre-warmed cell culture medium without FBS.
Note: Perform all wash steps carefully to maintain cell integrity and attachment. Low-velocity fluid dispensing is recommended.
6. Aspirate culture medium and add 200 µl of prepared VitalDye/DeadDye Solution (or individual prepared dyes) to each well. Incubate for 15 minutes at 37°C with 5% CO₂.
7. Aspirate dye solution and add 200 µl cell culture medium without FBS. Incubate for 5 minutes at 37°C with 5% CO₂.
8. Aspirate culture medium and add 200 µl/well of Fixation/Hoechst Solution. Incubate for 10 minutes at room temperature.
9. Aspirate Fixation/Hoechst Solution and wash plate twice with 1X Wash Buffer (at room temperature), leaving buffer from last wash in wells. Seal plate.
10. Evaluate plate on the ArrayScan Reader.
11. Store sealed plates in the dark at 4°C.

Additional Information

A. Dose-Response Curve and Assay Performance

HeLa cells were exposed to increasing concentrations of the positive control compound, and then labeled with VitalDye/DeadDye, as described in the protocol (Figure 2). HeLa cells grown in 96-well plates were processed using the Cell Viability Kit. The plate was evaluated on the ArrayScan Reader using the Cell Viability Application software (Figure 3).

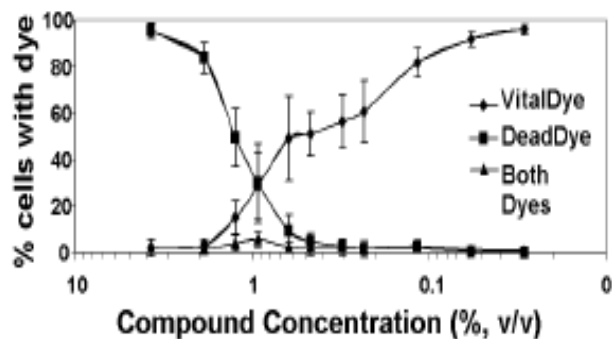


Figure 2. Dose-response curve of HeLa cells treated with the positive control compound. The percentage of cells containing the fluorescent dyes corresponds to the percentage of live (VitalDye) or dead (DeadDye) cells at increasing positive control concentrations.

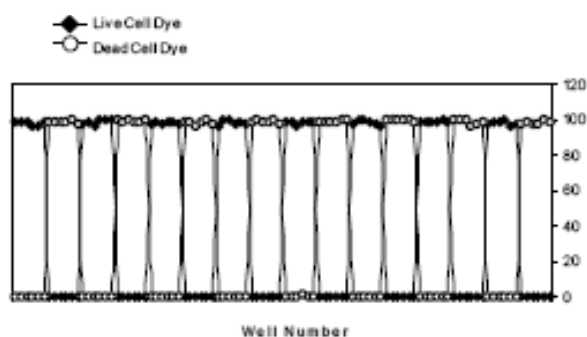


Figure 3. Cell viability assay performance. Columns 7-12 were treated with Tween-20 and columns 1-6 were untreated. The percentage of cells stained with VitalDye and DeadDye (y-axis) is shown for each well (x-axis).

B. Microscope Information

Cells prepared and labeled according to these instructions can be used and analyzed by fluorescence microscopes using the appropriate filter set(s) or confocal microscopy. Optimization may be required when using slides, coverslips or multi-well chamber slides. Use image-processing software to quantify the targets. The approximate absorption/emission maxima of the fluorescent dyes are as follows:

DeadDye = 535/617 nm

VitalDye = 492/516 nm

Hoechst Dye = 350/461 nm

C. Recommendations for Automation

- **Plating Cells:** To improve the uniformity and throughput of plating cells, use a liquid handling system such as Thermo Scientific Multidrop[®] Combi or WellMate[®] Dispensers.
- **Dead Volumes:** Every piece of automation instrumentation has a non-recoverable dead volume associated with it. Be aware of these dead volumes, priming volumes and rinsing volumes when calculating your reagent requirements.
- **Nonspecific Binding:** Because of the potential of reagent interaction with large surface areas inherent to tubing, syringes and peristaltic pumps, pre-priming with reagents or pre-coating with protein blockers may be warranted.
- **Mixing:** Gentle mixing may be required when adding a DMSO-based solution to keep overly concentrated solutions from lying on top of the cell layer. Be careful not to dislodge cells or beads during mixing procedures.
- **Cell Washing:** Use an automated plate washer designed to gently wash attached cells. Be careful not to dislodge cells or beads during cell washing.
- **Incubation:** Minimize the time when plates with live cells are out of a controlled CO₂ environment. For best results, use an automated incubator to deliver plates to a pipetting deck.
- **Exposure:** Minimize operator exposure to fixative by some form of containment. Some reagents and compounds are light-sensitive; be aware of these constraints when scaling up for an automated run.

- Adapting to other plate formats: When using different plate types, adjust reagent volumes as needed. Some suggested starting volumes are listed in Table 1.

Table 1. Suggested volumes to use for different cell culture plates.

<u>Kit Component</u>	<u>96-Well Plates</u> (μ l/well)	<u>384-Well Plates</u> (μ l/well)	<u>24-Well Plates</u> (μ l/well)
Fixation Solution	100	25	400
1X Wash Buffer	100	25	400
1X Blocking Buffer	100	25	400
1X Permeabilization Buffer	100	25	400
Antibody Solution	50	12.5	200
Staining Solution	50	12.5	200
1X Wash Buffer (final wash)	150	37.5	200

Compatible BioApplication Software Modules

S50-0003-1

Cell Viability BioApplication

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Thermo Scientific Cellomics Reagent Kits are developed and manufactured at the same Thermo Fisher Scientific Inc. facility as Pierce Protein Research Products and are supported by Pierce Technical Support (see contact information in page footer). These kits are part of the Cellomics Total Solution Platform for HCS, which also includes Cellomics ArrayScan and other HCS Instrumentation, BioApplication Image Analysis Software and High-Content Informatics. For more information, visit www.thermo.com/cellomics or call 800-432-4091 (toll free) or 412-770-2500.

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