

Cellomics[®] Multiparameter Cytotoxicity 1 Kits

High-Content Screening Reagents

1824.2

Number	Description
K0200021	Multiparameter Cytotoxicity 1 Kit , sufficient materials for 5 × 96 wells
R0200061	Multiparameter Cytotoxicity 1 Kit , sufficient materials for 50 × 96 wells

Kit Contents:	K0200021
MPCT1 Fluor Solution (100 X)	5 x 80 µl
Wash Buffer (10X)	100 ml
Thin Plate Seal Assembly	7/pack

Storage: Upon receipt store MPCT1 Fluor Solution protected from light at -20 °C. Keep remaining kit components at 4°C. 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 kit reagents are not for diagnostic use in humans or animals.

Introduction

The Multiparameter Cytotoxicity 1 Kit includes reagents for identifying changes in nuclear morphology and size,¹ membrane permeability status,² lysosome mass/pH changes³ and cell density changes⁴ (number of cells per field) caused by compound toxicity. When used with the Multiparameter Cytotoxicity 1 BioApplication software on the Thermo Scientific ArrayScan[®] HCS Reader, the kit can be used as a cell health assay where, for nuclear morphology, cell permeability and lysosomal physiology parameters, the number and percentage of cells in the population that are beyond the normal physiological range can be quantified. Additionally, the assay can be used to identify compounds affecting cell population density.

Depending on the type of toxic insult, cells often undergo either necrosis or apoptosis, accompanied by changes in nuclear size or morphology.^{5,6} The Hoechst 33342 dye used in this assay labels DNA and emits a blue fluorescence. This nuclear stain identifies individual nuclei and measures changes in nuclear size and morphology that result from toxic insult (Figure 1).

The cell membrane maintains cellular homeostasis, providing a specialized environment different from its extracellular surroundings, and providing a mechanism for the controlled exchange of its nutrients with its surroundings. Certain toxins can affect cell membrane integrity leading to the cell becoming permeable,⁷⁻⁹ eventually causing cell death. Healthy cells are nearly impermeable to the indicator dye used in this assay; however, after compromising the cell membrane's permeability, the dye stains the nucleus with a green fluorescence (Figure 2).

Physiologically healthy cells have intracellular organelles with characteristic properties, including lysosomes and endosomes that maintain specific internal acidic pH ranges for cells to function normally. Toxins can interfere with cell functionality by affecting the pH of these organelles or by causing a change in lysosome number.^{10,11} The dye used in the assay to determine changes to lysosomal physiology is a weak base that accumulates in acidic organelles such as lysosomes and endosomes. A decrease in pH of acidic organelles or increase in lysosome numbers by compound toxicity results in an increase of fluorescence intensity. Conversely, an increase in pH of these organelles or decrease in lysosome numbers of results in a decrease in staining intensity of the dye (Figure 3).

After labeling, cells are fixed with formaldehyde and scanned and analyzed with the ArrayScan HCS Reader and the Multiparameter Cytotoxicity 1 BioApplication software. The assay was developed, optimized and validated in the human hepatoma cell line HepG2. In addition to the liver and kidney, other target organs/tissues for xenobiotics (toxicity effects) could be the lungs, central nervous system, heart, immune system and even the skin. With minor protocol changes, this assay can be easily adapted for use with any cell types/lines that originate from the organs/tissues listed.

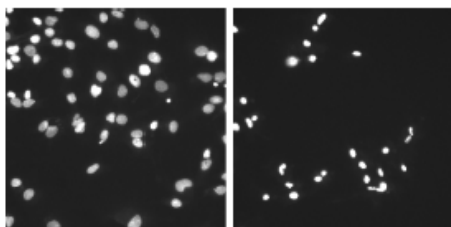


Figure 1. HepG2 cells stained for changes in nuclear size/morphology. Left panel: Non-treated cells. Right panel: Cells treated with 200 μM of valinomycin for 24 hours.

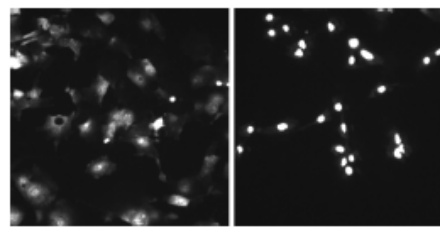


Figure 2. HepG2 cells stained for membrane permeability. Left panel: non-treated cells. Right panel: cells treated with 200 μM of valinomycin for 24 hours.

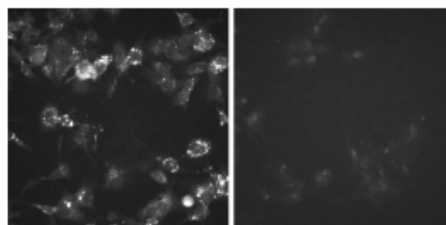


Figure 3. HepG2 cells stained for lysosomes. Left panel: non-treated cells. Right panel: cells treated with 200 μM of valinomycin for 24 hours.

Additional Materials Required

For the screening size kit, Wash Buffer is available separately. Please call customer service for more information.

- Formaldehyde (16%) (Thermo Scientific 16% Formaldehyde, Product No. 28906)
- Valinomycin (Sigma, Product No. V0627) or other cytotoxicity inducer
- Packard View 96-well microplates (Perkin-Elmer, Product No. 6005182)

Cell Preparation Information

- The protocol is optimized for HepG2 cells (ATCC, Product No. HB-8065)
- Culture cells in Minimum Essential Medium-Eagle (EMEM; BioWhittaker, Product No. 12-611Q) containing the following supplements (=EMEM Complete Medium): 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 solution (BioWhittaker, Product No. 13-115E), 1% penicillin/streptomycin solution (BioWhittaker, Product No. 17-602E)
- Split cells when they reach 70-80% confluency (every 3-4 days) at a dilution of 1:5.
- Harvest cells with trypsin-versene mixture (BioWhittaker, Product No. 17-161F), dilute into EMEM Complete Medium, and determine cell density.
- Dilute cells to 8×10^4 cells/ml in EMEM Complete Medium and add 100 μl of the cell suspension to each well of a 96-well microplate (= 8,000 cells/well).
- Incubate cells for 18 to 24 hours at 37°C in 5% CO_2 .

Multiparameter Cytotoxicity Kit Protocol

A. Solution Preparation (per 96-well plate)

1X Wash Buffer	Add 20 ml of 10X Wash Buffer to 180 ml of ultrapure water. Store diluted solution at 4°C for up to 7 days.
Fixation Solution	Add 2.2 ml of 37% formaldehyde to 19.8 ml of 1X Wash Buffer. Warm to 37°C before use. Prepare just before each assay.
1X MCPT1 Fluor Solution	Thaw one vial of 100X MPCT1 Fluor Solution in a water bath and centrifuge at maximum speed for 15 seconds. Dilute 65 µl of the 100X MPCT1 Fluor Solution to 6.5 ml in complete EMEM. Mix well and warm to 37°C before use. Prepare just before each assay.
Valinomycin Stock	Add 2.25 ml of DMSO directly to the 25 mg vial of valinomycin (Sigma, Product No. V0627) to make a 10 mM stock solution. Prepare 0.2 ml aliquots in plastic vials and store at -20°C until use.
Valinomycin Working Solution	Dilute the 10 mM Valinomycin Stock to 600 µM in EMEM complete medium.
0.01% Sodium Azide	Dilute a 10% stock solution of sodium azide (made in ultrapure water) to a final concentration of 0.01% in 1X Wash Buffer.

B. Procedure

Note: The MPCT1 Fluor Solution contains the following components:

- Hoechst Dye (Ex/Em wavelength: 350/461 nm) for labeling nuclei.
 - Cell permeability indicator (Ex/Em wavelength: 491/509 nm) for labeling nuclei of permeabilized cells.
 - Fluorescent weak base (Ex/Em wavelength: 577/599 nm) for labeling acidic organelles and measuring pH and mass.
1. Add 50 µl/well of the 600 µM Valinomycin Working Solution (or other cytotoxicity inducer). Incubate for 23.5 hours at 37°C.
 2. Add 50 µl pre-warmed 1X MPCT1 Fluor Solution to each well. Incubate for 30 minutes at 37°C.
 3. Aspirate medium and add 100 µl pre-warmed Fixation Solution to each well. Incubate in a fume hood at room temperature for 15 minutes. Prewarming fixative is critical to maintaining cell integrity.
 4. Aspirate Fixation Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.

Note: For long-term storage (> 1 day) of processed plates, add 0.01% sodium azide (not provided) to the Wash Buffer used in step 5, and store in the dark at 4°C.
 5. Aspirate Wash Buffer and add 200 µl/well of 1X Wash Buffer.
 6. Seal plate and immediately evaluate on the ArrayScan HCS Reader.

Additional Information

A. Assay Performance

For the dose-response curves, HepG2 cells were stimulated with valinomycin for 24 hours then labeled and fixed as described in the procedure (Figure 4). For the time-course experiment, HepG2 cells were stimulated with 100 μ M valinomycin for 0-24 hours and labeled and fixed as described in the procedure (Figure 5).

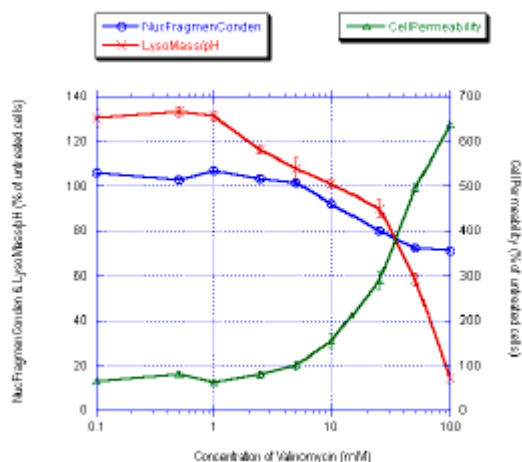


Figure 4. Dose-response curves for three cytotoxicity parameters of HepG2 cells induced by valinomycin. The EC₅₀ values were 16 μ M (Nuc FragConden), 25 μ M (cell permeability) and 43 μ M (Lyso Mass/pH). Response of control or untreated cells is set as 100%.

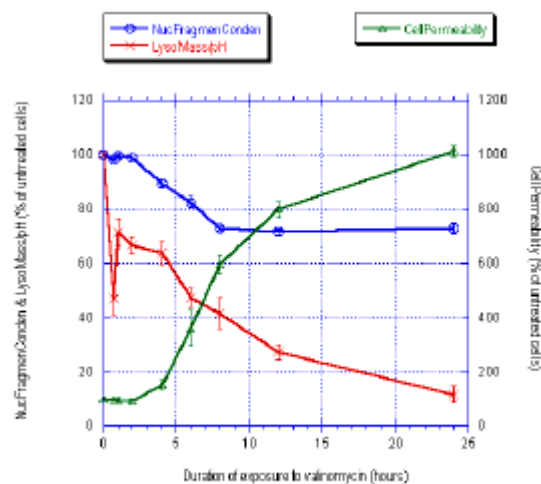


Figure 5. Time-course experiments of three cytotoxicity parameters of HepG2 cells induced by valinomycin. Response of control or untreated cells is set as 100%.

B. Microscope Information

Cells prepared and labeled according to this kit protocol can be used and analyzed by fluorescence microscopes using the appropriate filter set(s) or confocal microscopy. Optimization may be required when using slides, cover slips or multi-well chamber slides. Use image-processing software to quantify the targets.

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>	<u>384-Well Plates</u>	<u>24-Well Plates</u>
	(<u>µl/well</u>)	(<u>µl/well</u>)	(<u>µl/well</u>)
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

S50-0004-01 Multiparameter Apoptosis 1 BioApplication Software

References

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