For life science research only.
Not for use in diagnostic procedures.

Cell Viability Imaging Kit

Version: 06
Content Version: December 2020

Cat. No. 06 432 379 001 1 kit
5 × 96 reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	Cell Viability Imaging Kit, Nuclei Dye	Hoechst 33342	1 vial, 40 µl
2	red	Cell Viability Imaging Kit, Dead Dye	Propidium iodide	1 vial, 150 µl
3	green	Cell Viability Imaging Kit, Viable Dye	▪ Calcein-AM ▪ Solid	1 vial

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Nuclei Dye	Store dry at −15 to −25°C. ⚠ ***Keep protected from light.*** ⓘ *Solutions can be frozen and thawed at least 5 times.*
2	red	Dead Dye	
3	green	Viable Dye	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard cell culture equipment, such as a biohazard hood, +37°C incubator, centrifuge
- Standard pipettes and micropipettes
- Regular flat, clear bottom 96-well microplate
- Fluorescence microscope equipped with the appropriate excitation and emission filters

Standard laboratory reagents

- 1x phosphate buffered saline (PBS), cell-culture grade
- Dimethylsulfoxide (DMSO), cell-culture grade
- Culture medium

1.4. Application

The Cell Viability Imaging Kit provides for a simple and rapid method for viability measurement of mammalian cells using fluorescence detection.

- Total cell number, as well as the number of viable and dead cells are determined in parallel.
- Determination of total cell number uses nuclei staining with Nuclei Dye that stains the nuclei of both live and dead cells.
- The percentage of dead cells is detected based on membrane permeability using the Dead Dye.
- For confirmation, viable cells are also stained with the Viable Dye.

1.5. Preparation Time

Assay Time

For 1 × 96 well microplate

35 minutes

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use the following sample materials with the Cell Viability Imaging Kit:
- Adherent cells
- Suspension cells
- Cells treated with a concentration range of a cytotoxic agent, such as DMSO.

General Considerations

Precautions

When working with biological research samples:
- Always wear powder-free protective gloves.
- Handle all biological material as potentially infectious.
- Handling and disposal of potentially infectious biological material must be performed according to local safety guidelines.
- Disinfect spills immediately with an appropriate disinfectant solution to avoid spreading infection to, and contamination of, laboratory personnel and equipment.

Safety Information

Take special precautions when working with biological research samples:
- Always wear protective gloves (powder-free).
- Handle all biological material as potentially infectious.
- Handling and disposal of potentially infectious biological material should be performed according to local safety guidelines.
- Spills should be immediately disinfected with an appropriate disinfectant solution to avoid spreading infection to, and contamination of, laboratory personnel and equipment.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Cell culture conditions

Minimize intra- and inter-experimental variance by using cells that are regularly passaged, proliferating well (best when in a log-phase growth), and plated at a consistent density.

Preparation of adherent or suspension cells for the assay

1. Plate cells in appropriate cell culture medium using a regular flat, clear bottom, 96-well microplate, adding 150 µl per well.
   – In the case of adherent cells, cell confluence at time point of treatment should not be >50%.
   – For suspension cells, do not add more than 10,000 cells to each well.

2. Induce cytotoxicity, for example, by adding a cytotoxic compound in a dose-dependent manner.

3. Stain cells as described in section, **Staining and analyzing cells**.

Staining and analyzing cells

1. Prepare the Viable Dye solution:
   – Centrifuge the Viable Dye (Vial 3) for 15 seconds in a microcentrifuge to collect all substance at the bottom of the vial.
   – Pipette 25 µl DMSO into the vial.
   – Vortex well to dissolve the contents of the vial and briefly centrifuge again to collect all of the liquid at the bottom of the vial.
   The remaining Viable Dye solution can be stored at −15 to −25°C and then thawed for additional experiments as described in Step 2.

2. Briefly vortex the vials after thawing.
   – Allow all reagents to equilibrate to +23 to +27°C.
   Protect vials from exposure to light throughout the procedure.

3. Dilute 5 µl of Nuclei Dye (Vial 1) in 5 ml, 1x cell-culture grade PBS and vortex.

4. Add 25 µl of Dead Dye (Vial 2) to the mixture from Step 3 and vortex.

5. Add 3 µl of Viable Dye (Vial 3) to the mixture from Step 4 and vortex.

6. Pipette 50 µl of dye mixture from Step 5 into each well of the microplate.
   – Immediately after adding, briefly stir the dye mixture and media in the wells with the pipette tips to distribute the mixture evenly in the wells.
   For adherent cells, be careful when pipetting the dye mixture into the wells to ensure that the cells remain attached to the microplate.

7. Incubate the microplate for 30 minutes at +37°C.

8. Analyze the plate with regard to cell count and viability using a suitable fluorescence microscope with the appropriate excitation and emission filters.
   – Alternatively, use an appropriate system for automated image acquisition and analysis or detection by flow cytometry.

2.3. Parameters

Emission

Vial	Label	Emission Maximum [nm]
1	Nuclei Dye	486
2	Dead Dye	617
3	Viable Dye	515

Excitation Maximum

Vial	Label	Excitation Maximum [nm]
1	Nuclei Dye	361
2	Dead Dye	535
3	Viable Dye	490

3. Results

DMSO-induced cytotoxicity

Results in Figure 1 show HeLa cells treated with various concentrations of DMSO, and then processed as indicated below:

1. Plate HeLa cells in a standard, 96-well microplate using 5,000 cells in 150 µl cell culture media per well.

2. Incubate the microplate for 24 hours in a tissue-culture incubator at +37°C, 5% CO_2, and 95% relative humidity.

3. Twenty-four hours after seeding the cells in the microplate, add 50 µl of different concentrations of DMSO diluted with culture media to each well.
   – In the example shown in Figure 1, final DMSO concentrations in the wells were 1%, 4%, 7%, and 10%; in addition, a control using culture media without DMSO was included.

4. Incubate for 24 hours and proceed as described in section, **Staining and analyzing cells**.

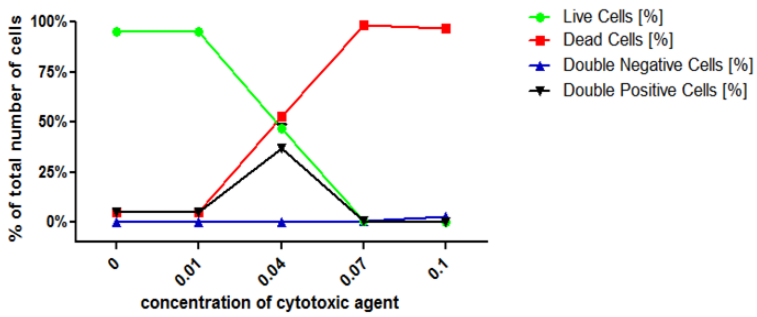

Fig. 1: Results of analysis of DMSO-induced cytotoxicity in HeLa cells using the Cell Viability Imaging Kit together with a Cellavista Analyzer.

4. Troubleshooting

Observation	Possible cause	Recommendation
Labeling too intense.	Dye too highly concentrated.	Try different, more dilute, dye concentrations.
Low labeling occurs.	Dye concentration too low.	Increase concentration of the dye in the wells.
	Incubation time too short.	Increase the incubation time.
	Fluorescence bleaching may occur upon prolonged exposure to bright light.	Keep samples in the dark after staining.
High background present.	Compound added shows autofluorescence.	Carefully wash out the compound before performing the assay.
	Mycoplasma contamination present.	Use the Mycoplasma PCR ELISA Kit* to check for possible mycoplasma contamination.
Nonspecific labeling occurs.	Samples left too long in dye mixture before analysis performed.	Perform analysis with a microscope immediately after the incubation step.

5. Additional Information on this Product

5.1. Test Principle

How this product works

- Hoechst 33342 is a cell-permeable stain that binds to DNA and can be used for staining nuclei of living or dead cells.
- Propidium iodide is a fluorescent nucleic-acid dye that can only pass through damaged dead cell membranes. It cannot pass through a viable cell membrane.
- Calcein-AM is cell membrane permeable, but not by itself a fluorescent molecule. Esterase found in viable cells interacts with Calcein-AM to produce calcein, a fluorescent molecule. Only viable cells are stained after treatment with Calcein-AM.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Trademarks

All product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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