

instaCELL cytotoxicity assay kit

Product Information

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1 Description

Cytotoxicity tests are commonly used in various fields, including pharmaceuticals, medical devices, cosmetics, and chemicals, to evaluate the safety of substances and materials. By measuring the cytotoxic effects of a substance *in vitro*, it is possible to determine if it has the potential to harm living tissues or organs. This information is crucial for identifying and eliminating or modifying potentially toxic compounds, thereby ensuring the safety of products and protecting human health.

The instaCELL cytotoxicity assay kit is a fluorometric cell viability assay to assess the metabolic activity and cell viability of living cells. The assay is based on the reduction of a redox indicator called resazurin to a fluorescent and colorimetric compound called resorufin by metabolically active cells. Resazurin is a non-toxic, cell-permeable dye that enters living cells and changes its color and fluorescence properties upon reduction by viable cells. The amount of resorufin produced is directly proportional to the metabolic activity of the cells, which, in turn, reflects their viability.

Commonly, these tests are conducted with cells that have been maintained in culture for several passages to ensure optimal cell fitness. However, in recent years it has been demonstrated for various applications that cryopreserved cells can be used instantly after thawing when optimized freezing protocols are applied. Assay ready L-929 cells provide a robust tool to test cytotoxicity. Instantly after thawing, the cells display the same sensitivity to toxic reference controls as cells from a continuously passaged maintenance culture.

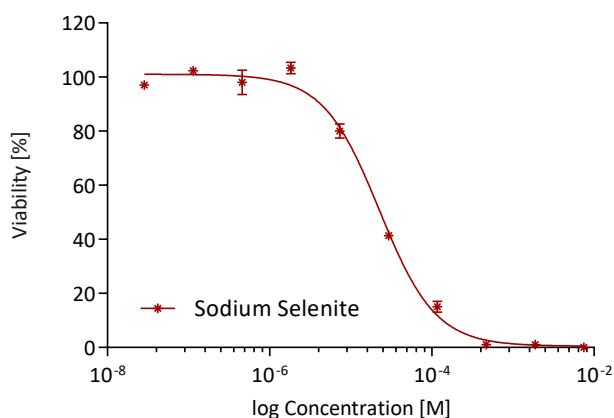


Figure 1: Dose-Response curve of the Cytotoxic Control used in the instaCELL cytotoxicity assay kit.

2 Cell Information

Cell Type:	Fibroblast
Tissue:	Connective Tissue
Species:	Mouse
Growth:	Adherent
Gender:	Male
Biosafety Level:	1

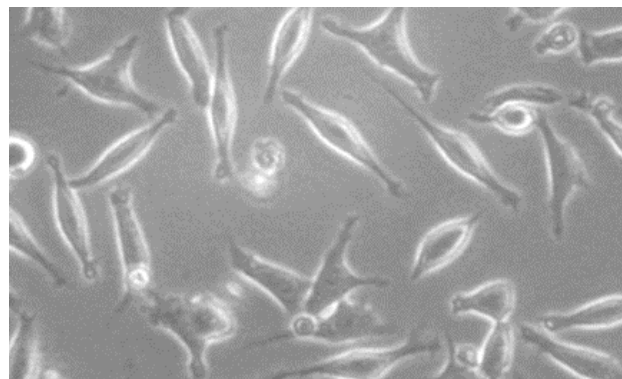


Figure 2: Morphology of Assay Ready L-929 Cells

3 Kit Content

• L-929 cells	1 vial (1.0E+07 cells)	RE772
• Recovery Buffer A	1 bottle (10ml)	MD163-01
• Assay Buffer A	1 bottle (60ml)	MD363-06
• Assay Medium A	1 bottle (10ml)	MD463-01
• Resazurin	1 bottle (5ml)	RX718-01
• Cytotoxic Control (Sodium Selenite)	1 vial (15mM)	RX501-01
• 96-well plate	1 plates	83.3924.300 (Sarstedt)

Additionally required but not provided with the kit:

15ml centrifuge tube, PBS

4 Protocol of Use

4.1 day I: preparation of cells and samples

- Equilibrate all media and buffer to 37°C.
- Keep the cells on dry ice before thawing and process quickly.
- Thaw assay ready cells in a water bath at 37°C for 2min.
- Prepare 9ml of recovery buffer in a 15ml centrifugation tube (not provided). Transfer the cells completely into the prepared tube.
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant. Resuspend the cell pellet in 10ml of assay medium.
- Prepare appropriate dilutions of your test sample in assay buffer at 2x of the final concentration.
- Dispense 80µl of cell suspension into each well of the provided 96-well plate. Make sure to mix the suspension in-between to ensure an even distribution of the cells.
- Add 80µl of the 2x sample dilutions and of the Cytotoxic Control in triplicates into the corresponding wells.
- Incubate for 24h in a humidified incubator at 37°C and 5% CO₂.

4.2 day II: staining and read-out

- Add 20µl of resazurin solution to each well of the assay plate. Mix by moving the plate crosswise or use a plate shaker.
- Incubate the cells for 4h in a humidified incubator at 37°C and 5% CO₂. Viable cells will metabolize the blue resazurin to fluorescent, pink colored resorufin.
- Mix by moving the plate crosswise or use a plate shaker before measurement.
- Measure the fluorescence in plate reader at 540nm/590nm to determine the viability of the cells.

5 Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	X	X	X	X	X	X	X	X	X	X	X
B	Solvent	comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 1 C6	comp 1 C7	comp 1 C8	comp 1 C9	comp 1 C10	Solvent
C	Solvent	comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 1 C6	comp 1 C7	comp 1 C8	comp 1 C9	comp 1 C10	Solvent
D	Solvent	comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 1 C6	comp 1 C7	comp 1 C8	comp 1 C9	comp 1 C10	Solvent
E	Solvent	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	comp 2 C6	comp 2 C7	comp 2 C8	comp 2 C9	comp 2 C10	Solvent
F	Solvent	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	comp 2 C6	comp 2 C7	comp 2 C8	comp 2 C9	comp 2 C10	Solvent
G	Solvent	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	comp 2 C6	comp 2 C7	comp 2 C8	comp 2 C9	comp 2 C10	Solvent
H	X	X	X	X	X	X	X	X	X	X	X	X

■ test substance 1 (triplicates of 10 dilutions), ■ test substance 2 (triplicates of 10 dilutions), ■ solvent control, X blank (Assay Buffer)

6 Analysis and Evaluation

To calculate the viability, use the equation below:

$$Viab.\% = \frac{100 \times RFU_{sample} - RFU_{blank}}{RFU_{solvent} - RFU_{blank}}$$

If the viability is reduced to <70% of the solvent control (SC), the extract has a cytotoxic potential.

7 Stability & Storage

Performance of the kit is guaranteed within the specifications as defined in the certificate of analysis only before the expiration date as indicated on the packaging and only if stored and handled according to the instruction of this datasheet.

- Store Assay Ready Cells below -140°C (e.g., in vapor phase of liquid nitrogen).
- Store all other reagents and media as indicated on the label.

8 Literature & Related Documents

[1] ISO 10993-5 Biological evaluation of medical devices – Tests for *in vitro* cytotoxicity

9 Support

<https://www.accelerate.me/support/contact.html>

Phone: +49 (40) 33 464 73 20

10 Disclaimer

The product is sold under the terms of a Limited Use Label License attached to the product. By breaking the seal of the product package, the user explicitly agrees to the license terms. Assay Ready Cells are for immediate assay use only. The user shall not propagate, passage, or refreeze the cells.

This product is intended for research use only. Do not use for diagnostic or therapeutic purposes.