instaCELL cytotoxicity assay kit (XTT)

Product Information

Cat N° SF020-02

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Content

1 Description

Figure 1: instaCELL cytotoxicity assay kit (XTT)

Cytotoxicity tests are commonly used in various fields, including pharmaceuticals, cosmetics, and chemicals, to evaluate the safety of substances. 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.

Medical devices must be reviewed for their proper function as well as for the compatibility of the devices with surrounding tissue. One aspect of this biocompatibility testing is the determination of the cytotoxicity of an extract prepared from a device's material as explained in part 5 of the ISO 10993 guideline [1]. Immortalized mammalian cells are incubated *in vitro* with the extracts for a certain time and cell viability is determined by using dyes like XTT, which are metabolized in living cells.

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 of medical devices according to ISO 10993-5. Instantly after thawing, the cells display the same sensitivity to extracts prepared from toxic reference material as cells from a continuously passaged maintenance culture (Fig. 2).

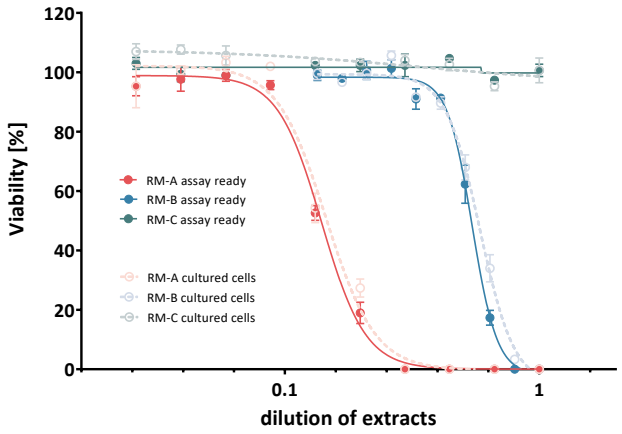

Figure 2 Dose dependent toxicity of reference extracts tested on cultured versus assay ready frozen L-929 cells.

2 Cell Information

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

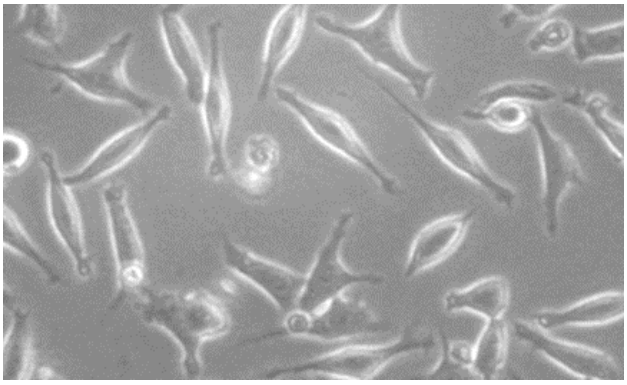

Figure 3: Morphology of Assay Ready L-929 Cells

3 Kit Content

• L-929 cells	1 vial (3E+06 cells)	RE772K
• Recovery Buffer A	1 bottle (10ml)	MD163-01
• Assay Buffer A	1 bottle (60ml)	MD363-06
• Assay Medium A	1 bottle (60ml)	MD463-06
• PMS Solution	1 vial (25µl)	RX740-01
• XTT Solution	1 bottle (6ml)	RX758-06
• Cytotoxic Control	1vial (1.8ml)	RX501-01
• 96-well plate	2 plates	83.3924.300 (Sarstedt)

Additionally required but not provided with the kit:

15ml centrifuge tube

Related Products:

- RX520-01 Biocompatibility Reference Material

4 Protocol of Use

For medical device testing, use the biocompatibility reference material (RX520-01) and start the extraction at day I, parallel to preparation of the cells.

4.1 day I: preparation of cells

- Equilibrate all media and buffer to room temperature.
- 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 15ml of assay medium.
- Dispense 100µl of cell suspension into each well (except rows A and H) of the provided 96-well plate.
- Incubate for 24h in a humidified incubator at 37°C and 5% CO_2.

4.2 day II: addition of the test sample and controls

- Prepare appropriate dilutions of your test samples in assay buffer.
- Aspirate the culture medium from each well.
- Add 100µl of test sample, controls and blank to the respective wells of the recommended plate layout. Use Assay Buffer as blank control. Each test sample should be analyzed in at least four test concentrations in triplicates.
- Incubate for 24h in a humidified incubator at 37°C and 5% CO_2.

4.3 day III: staining and read out

- Complete the XTT solution by adding 15µl of PMS and mix well.
- Dispense 50µl of PMS/XTT into each well and incubate for 4h in a humidified incubator at 37°C and 5% CO_2
- Sway the plates carefully and transfer 100µl of the supernatant from each well into the corresponding well of a 96-well plate.
- Measure OD at a wavelength of 450nm and reference at 630nm.

5 Plate layout

5.1 Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
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												

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

5.2 Recommended plate layout for medical device testing

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		RM-A 100%	RM-A 50%	RM-A 25%	RM-A 12.5%	RM-B 100%	RM-B 50%	RM-B 25%	RM-B 12.5%	RM-C 100%	Solvent	
C		RM-A 100%	RM-A 50%	RM-A 25%	RM-A 12.5%	RM-B 100%	RM-B 50%	RM-B 25%	RM-B 12.5%	RM-C 100%	Solvent	
D		RM-A 100%	RM-A 50%	RM-A 25%	RM-A 12.5%	RM-B 100%	RM-B 50%	RM-B 25%	RM-B 12.5%	RM-C 100%	Solvent	
E		comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	
F		comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	
G		comp 1 C1	comp 1 C2	comp 1 C3	comp 1 C4	comp 1 C5	comp 2 C1	comp 2 C2	comp 2 C3	comp 2 C4	comp 2 C5	
H												

positive control 1 (triplicates of 4 dilutions), positive control 2 (triplicates of 4 dilutions), negative control

test substance 1 (triplicates of 5 dilutions), test substance 2 (triplicates of 5 dilutions), solvent control, X blank (Assay Buffer)

6 Analysis and Evaluation

To calculate the viability, use the equation below:

$$Viab.\% = \frac{100 \times OD_{450e}}{OD_{450b}}$$

OD_{450e} is the mean value of the test sample concentration

OD_{450b} is the mean value of the solvent control

If the viability is reduced to <70% of the solvent control (SC), the extract has a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract otherwise, the test should be repeated.

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.accellerate.me/support/contact.html

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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.