

kit content

- assay ready cells (1x vial)
- assay buffer A (1x bottle)
- assay medium A (1x bottle)
- recovery buffer A (1x bottle)
- 96-well assay plates (2x)
- XTT (1x bottle)
- PMS (1x vial)
- RM-A (ZEDC) (1x tube)
- RM-B (ZDBC) (1x tube)
- RM-C (PE) (1x tube)



not provided

- 15ml centrifugation tube

storage

- Store assay ready cells in liquid nitrogen (below -140°C)
- Store all reagents and media at -20°C

+

limited product warranty

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limited use license

Assay Ready Cells are provided under a limited use license and shall only be used instantly for the particular purpose of the instaCELL bioassay kit. No further expansion, passaging or refreezing of the cells is permitted. When breaking the sealed bag of the assay ready cells the user is explicitly accepting the terms of this limited use license.

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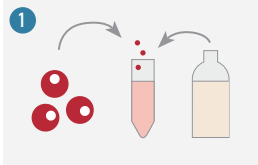
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instaCELL[®] biocompatibility assay kit protocol



SAFETY

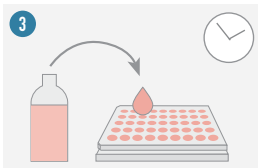
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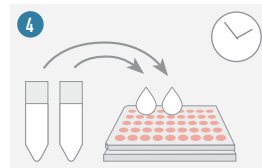
thaw cells for **2min** at **37°C**, dilute in **8ml** recovery buffer



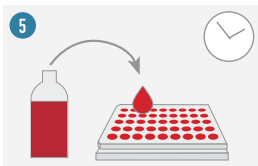
centrifuge for **3min** at **80xg**, resuspend in **10ml** assay medium



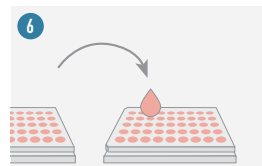
dispense cells **100 µl/well**, incubate for **1h**



aspirate medium, add **100 µl/well** of extract dilutions, incubate for **24h**



add **50 µl/well** of XTT/PMS, incubate for **4h**



transfer **100µl** of supernatant from each well into an empty plate



measure OD at **450nm** (ref. **630nm**)

day I: preparation of extracts

- Add **2ml** of assay buffer to each reference material provided in a **15ml** tube and extract for **24h** at **37°C** on a shaker (make sure the material is completely covered with medium).
- Prepare the extract of your test sample.

day II: preparation of cells and incubation with extracts

- 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 **8ml** of recovery buffer in a 15ml centrifugation tube (not provided). Transfer the cells completely into the prepared tube. **1**
- Centrifuge for **3min** at **80xg** and carefully aspirate the supernatant. Resuspend the cell pellet in **10ml** of assay medium. **2**
- Dispense **100µl** of cell suspension into each well (except rows A and H) of the provided 96-well plate. Incubate for **1h** in a humidified incubator at **37°C** and **5% CO₂**. **3**
- Prepare two-fold dilutions of the test sample extracts and reference extracts in assay buffer (see plate layout).
- Carefully aspirate the supernatant. Add **100µl** of the extract dilutions to individual wells of the plate (see plate layout). Incubate for **24h** at **37°C** and **5% CO₂**. **4**

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% CO₂**. **5**
- Sway the plates carefully and transfer **100µl** of the supernatant from each well into the corresponding well of a 96-well plate. **6**
- Measure OD at a wavelength between **450-500nm** and reference between **630-690nm**. **7**
- If the viability is reduced to **<70%** of the medium control (M), the extract has a cytotoxic potential. To calculate the viability use the equation below:

$$\text{Viab. \%} = \frac{100 \cdot \text{mean OD}_{450e}}{\text{mean OD}_{450b}}$$

	1	2	3	4	5	6	7	8	9	10	11	12
A	M	RM-A 1:1	1:2	1:4	1:8	1:16	RM-B 1:1	1:2	1:4	1:8	1:16	M
B	M	RM-A 1:1	1:2	1:4	1:8	1:16	RM-B 1:1	1:2	1:4	1:8	1:16	M
C	M	RM-A 1:1	1:2	1:4	1:8	1:16	RM-B 1:1	1:2	1:4	1:8	1:16	M
D	M	RM-A 1:1	1:2	1:4	1:8	1:16	RM-B 1:1	1:2	1:4	1:8	1:16	M
E	M	RM-C 1:1	C1	C2	C3	C4	C5	C6	C7	C8	C9	M
F	M	RM-C 1:1	C1	C2	C3	C4	C5	C6	C7	C8	C9	M
G	M	RM-C 1:1	C1	C2	C3	C4	C5	C6	C7	C8	C9	M
H	M	RM-C 1:1	C1	C2	C3	C4	C5	C6	C7	C8	C9	M

RM-A- moderate toxicity control
 RM-B- weak toxicity control
 RM-C- negative control

M - medium
 C1-C9- extract dilutions
 / - no cells