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

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instaCELL® biocompatibility assay kit
protocol

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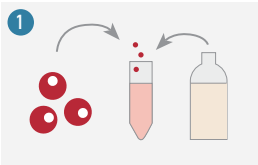
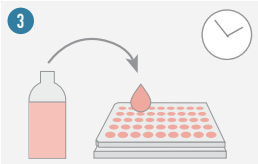
SAFETY

Version 1.0

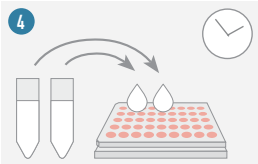


thaw cells for 2min at 37°C, dilute in 8ml recovery buffer

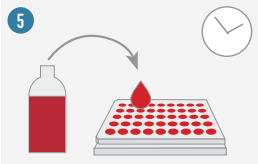
centrifuge for 3min at 80xg, resuspend in 10ml assay medium



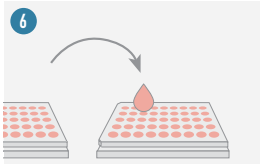
dispense cells 100 µl/well, incubate for 1h



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)

	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

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% CO_2. 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% CO_2. 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% CO_2. 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:

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