

instaCELL[®] biocompatibility assay kit protocol



Version 1.0
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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

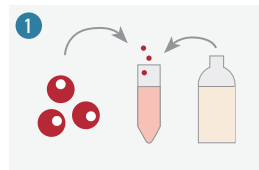
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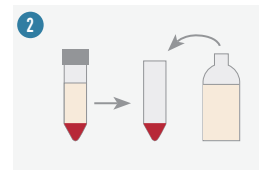
Assay Ready Cells are provided under a limited use license and shall only be used instantly for the particular purpose of the instaCELL bioassay kit. No further expansion, passaging or refreezing of the cells is permitted. When breaking the sealed bag of the assay ready cells the user is explicitly accepting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



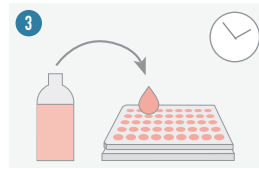
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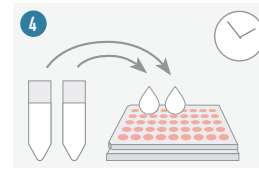
1 thaw cells for 2min at 37°C, dilute in 8ml recovery buffer



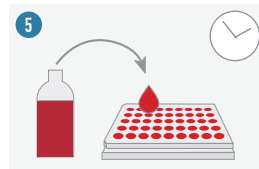
2 centrifuge for 3min at 80xg, resuspend in 10ml assay medium



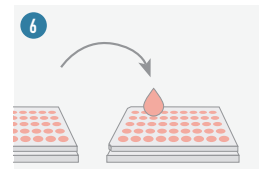
3 dispense cells 100 µl/well incubate for 1h



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



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



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



7 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 from your test sample in the same way.
- Prepare two-fold dilutions of the test sample extracts and reference extracts in assay buffer (see plate layout).

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
- 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	1:16	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	1:16	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	1:16	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	1:16	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

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