



SAFETY



# instaCELL<sup>®</sup> cytotoxicity assay kit

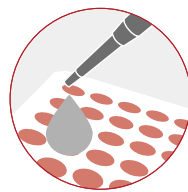
## XTT protocol



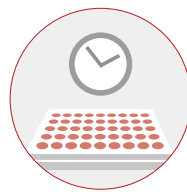
thaw



dispense



add samples



incubate



measure

Version 1.1

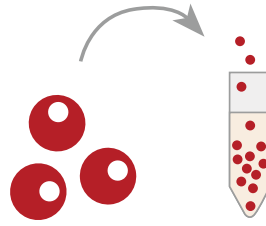
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1



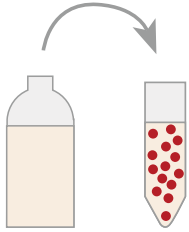
thaw cells  
*2 min*  
*37°C*

2



transfer  
cells into  
*15 ml tube*

3



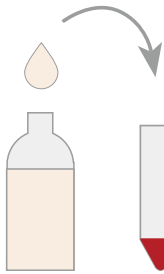
add  
washing  
buffer  
*8 ml*

4



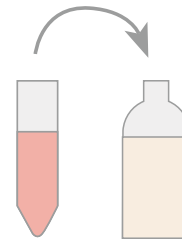
centrifuge  
*180 xg, 4 min*  
and discard  
supernatant

5



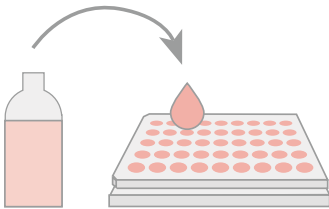
resuspend pellet  
in assay medium  
*4 ml*

6



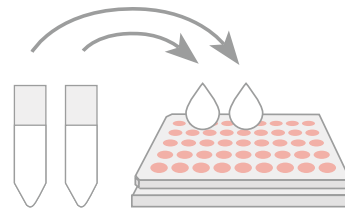
mix suspension  
to assay medium  
bottle

7



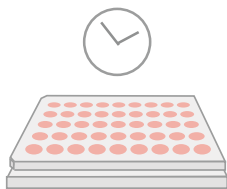
dispense  
into plates  
*80 µl*

8



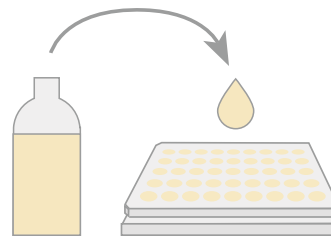
add 2x  
samples  
*80 µl*

9



incubate  
*24h*

10



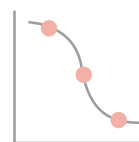
add XTT  
*20 µl*

11



incubate  
*4h*

12



measure absorbance  
*475 nm*

### **before the start:**

- Carefully read the protocol.
- Equilibrate all media, buffer and solutions to room temperature.
- Prepare appropriate dilutions of your test samples in assay buffer at 2x of the final assay concentration.

### **day one: preparation of cells and application of samples**

**! Cryopreserved cells are very fragile. Keep on dry ice before thawing and process quickly!**

- 1 Thaw a vial of Frozen Instant Cells in a water bath (37°C) for 2 minutes.
- 2 Transfer the cells completely into a 15 ml centrifugation tube (not provided).
- 3 Add 8 ml of washing buffer.
- 4 Centrifuge for 4 minutes at 180 xg. Carefully aspirate the supernatant.
- 5 Resuspend the cell pellet in 4 ml of assay medium.
- 6 Transfer suspension into the bottle with the remaining medium. Mix carefully.

**! The volume of assay medium is adjusted to each batch of cells. Make sure that each aliquot of cells is finally resuspended in a complete bottle of assay medium!**

- 7 Dispense 80 µl of the cell suspension into each well of a provided 96-well plate.

**! Cells sediment fast. Make sure to mix the suspension in-between to ensure an even distribution of the cells!**

- 8 Add 80 µl of 2x sample dilutions or toxic control to the wells.
- 9 Incubate the plate in a humidified incubator (5% CO<sub>2</sub>) at 37°C for 24 hours.

### **day two: performing the assay and read-out**

- 10 Add 20 µl of XTT solution to each well of the assay plate. Mix by moving the plate crosswise on the desk or use a plate shaker.
- 11 Return the plate to the incubator and incubate for another 4 hours. Viable cells will metabolize the yellow tetrazolium salt to a deep orange colored formazan.
- 12 Mix by moving the plate crosswise on the desk or use a plate shaker. Measure absorbance in a plate reader at 475 nm (690 nm reference).

## kit content

- Frozen Instant Cells (5x vials)
- recovery buffer (1x bottle)
- assay medium (5x bottles)
- assay buffer (1x bottle)
- XTT solution (5x bottles)
- toxic control (5x vials)
- 96-well assay plates (5x)
- protocol (1x)

## storage

Store Frozen Instant Cells in liquid nitrogen (below -150°C) and all reagents and media at -20°C to guarantee a shelf life of 6 month after delivery. If the cells are stored below -70°C the shelf life reduces to 4 weeks.



## limited product warranty

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by acCELLerate. acCELLerate shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product. The product is for research only and must not be used for diagnostic or therapeutic purposes.

## limited use license

Frozen Instant Cells are sold under a limited use licence 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 Frozen Instant Cells the user is explicitly accepting the terms of this limited use license.

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