

## Viability Assay

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**Overview:** This protocol is used to assess the viability of cells in a cell-laden 3D bioprinted construct. It is recommended to assess the viability the day after 3D bioprinting, and at selected time points throughout the cultivation of cell-laden constructs.

**Materials:**

- Invitrogen LIVE/DEAD Cell Imaging Kit 488/570 (Cat No R37601)
- Fluorescent Microscope with green and red imaging filters, set up to enable imaging
- Hank's Balanced Salt Solution (HBSS)
- Cell-laden printed constructs

**Protocol:**

1. Thaw LIVE/DEAD Imaging Kit Vials. Protect the working solution and stained cells from light sources
2. Remove cell-laden constructs from the incubator. Wash constructs: aspirate medium, add HBSS and incubate 15 min at 37°C
3. Transfer the contents of the LIVE green vial to the DEAD red vial. Add 1 ml HBSS to the vial and mix to obtain a 1x L/D working solution
4. Remove the cell-laden constructs from the incubator. Aspirate the HBSS and add the 1x L/D working solution to the cell-laden constructs, ensuring complete liquid coverage of the constructs. Incubate 1h at 37°C
5. Wash the constructs HBSS 2x 30 min at 37°C
6. Bring cell-laden constructs to fluorescent microscope for imaging
7. Capture images in the green (FITC/488) and red (TexasRed/570) channels. Assess the viability percentage using any software for image analysis, e.g. Image J.

**Further Information:**

This protocol is optimized based on CELLINK™ Bioink, and may need further optimization for other bioinks.

[Invitrogen LIVE/DEAD Cell Imaging Kit Protocol.](#)

**References:**

Markstedt K *et al.* 3D Bioprinting Human Chondrocytes with Nanocellulose-Alginate Bioink for Cartilage Tissue Engineering Applications. *Biomacromolecules*. 2015;16;1489-96  
Martínez H *et al.* 3D Bioprinting of Human Chondrocyte-laden Nanocellulose Hydrogels for Patient-specific Auricular Cartilage Regeneration. *Bioprinting*. 2016;1;22-35