

## Viability Staining Protocol

# INVITROGEN™

*This is a suggested procedure, please adjust according to your experimental needs.*

### Protocol aim

The aim of this protocol is to provide instructions for performing live dead staining using the Invitrogen LIVE/DEAD Cell Imaging Kit for imaging of live respectively dead cells in 3D constructs of CELLINK bioink, GelMA and GelX variations.

### Material needed

- Invitrogen LIVE/DEAD Cell Imaging Kit 488/570\*
- Hank's Balanced Salt Solution (HBSS+/-)
- Cell-laden printed constructs
- Inverted fluorescence microscope with filter sets for FITC and Texas Red
- Image analysis software

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\* Store stock solution at -20°C. Invitrogen LIVE/DEAD Cell Imaging Kit 488/570 can be purchased from Invitrogen™, Catalog Number R37601.

**KEEP STOCK, WORKING SOLUTION AND STAINED CELLS FROM LIGHT SOURCES.**


### Protocol

This protocol is optimized for CELLINK bioink, GelMA and GelX variations, printed in larger constructs of 10x10x5 mm. For other sizes and structures, please adjust volumes and incubation times. Larger constructs need larger staining solution volumes to fully cover the construct and longer incubation times for the dye to penetrate to the centre of the 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.

**TIME:** ~125 min until ready to image.

Step	Title	Material	Description
1	Preparation	- LIVE/DEAD Cell Imaging Kit	- Thaw LIVE/DEAD Imaging Kit Vials. <b>TIME:</b> ~5 min.

2	Wash	<ul style="list-style-type: none"> <li>- Cell-laden printed constructs</li> <li>- HBSS+/+</li> </ul>	<ul style="list-style-type: none"> <li>- Remove cell-laden constructs from the incubator. If wanted, take an extra sample to use as dead control.</li> <li>- Wash constructs by carefully aspirating medium and gently adding HBSS+/+.</li> <li>- Incubate 15 min at 37°C.</li> </ul> <p><b>TIME:</b> ~25 min.</p>
3	Prepare staining solution	<ul style="list-style-type: none"> <li>- HBSS+/+</li> </ul>	<ul style="list-style-type: none"> <li>- Transfer the contents of the LIVE green vial to the DEAD red vial.</li> <li>- Add 1 ml HBSS+/+ to the vial and mix to obtain a 1x LIVE/DEAD working solution.</li> </ul> <p><b>TIME:</b> ~5 min.</p>
4	Apply staining solution	<ul style="list-style-type: none"> <li>- 1x L/D working solution</li> </ul>	<ul style="list-style-type: none"> <li>- Remove the cell-laden constructs from the incubator.</li> <li>- Aspirate the HBSS+/+ and add the 1x LIVE/DEAD working solution to the cell-laden constructs, ensuring complete liquid coverage of the constructs.</li> <li>- Incubate 1h at 37°C</li> </ul> <p><b>TIME:</b> ~1h 10 min.</p>
5	Wash	<ul style="list-style-type: none"> <li>- HBSS+/+</li> </ul>	<ul style="list-style-type: none"> <li>- Wash the constructs with HBSS+/+ twice for 15 min at 37°C.</li> </ul> <p><b>TIME:</b> ~20 min.</p>
6	Imaging	<ul style="list-style-type: none"> <li>- Fluorescent Microscope</li> </ul>	<ul style="list-style-type: none"> <li>- Bring cell-laden constructs to fluorescent microscope for imaging.</li> <li>- Capture images in the green (FITC/488) and red (TexasRed/570) channels.</li> </ul>
7	Image analysis	<ul style="list-style-type: none"> <li>- Image analysis software</li> </ul>	<ul style="list-style-type: none"> <li>- Assess the viability percentage using a software for image analysis, e.g. Image J.</li> </ul>

 Want to see our talented Biologist proceed to this protocol? Feel free to find the video here: <https://www.youtube.com/watch?v=IJmDYfTaAy4>

### Photos of some applications:

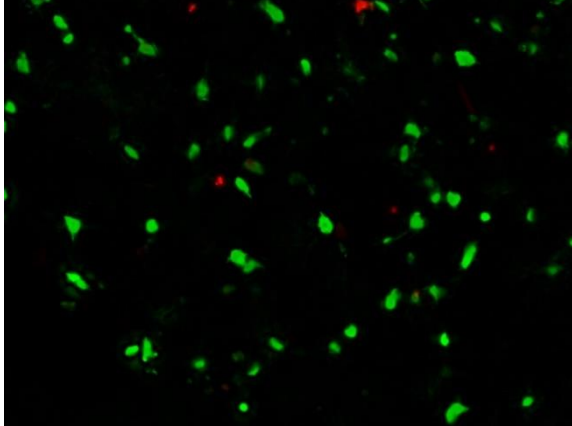


Figure 1. Epidermal side of SKIN construct day 14.

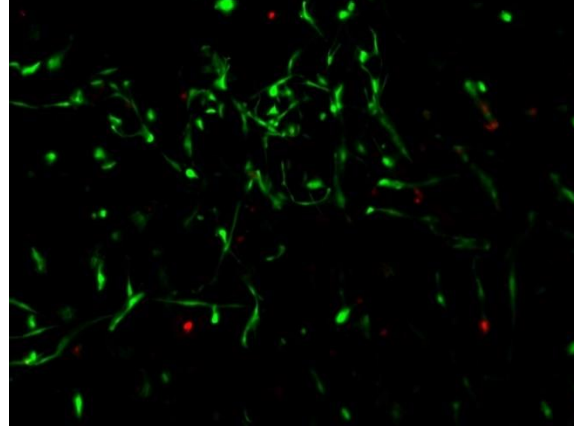


Figure 2. Dermal side of SKIN construct day 14.

Want to see our existing tissue model?



Just go to <http://bioverse.co/> and discover a whole library of CAD files especially created for sharing 3D Bioprinting models.

### References:

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