

CELLINK SKIN+ VIABILITY ASSAY

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

Aim of the protocol:

To assess the viability of cells in a cell-laden 3D bioprinted construct.

Remark:

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 needed:

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

Protocol:

Step n°	Title	Material	Description
1	Preparation	<ul style="list-style-type: none"> ▪ LIVE/DEAD Cell Imaging Kit 	<ul style="list-style-type: none"> ❖ Thaw LIVE/DEAD Imaging Kit Vials. ❖ Protect the working solution and stained cells from light sources
2	Wash	<ul style="list-style-type: none"> ▪ Cell-laden printed constructs ▪ HBSS 	<ul style="list-style-type: none"> ❖ Remove cell-laden constructs from the incubator ❖ Wash constructs: <ul style="list-style-type: none"> - aspirate medium - add HBSS ❖ Incubate 15 min at 37°C
3	Prepare staining solution	<ul style="list-style-type: none"> ▪ HBSS 	<ul style="list-style-type: none"> ❖ 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

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4	Apply staining solution	<ul style="list-style-type: none"> 1x L/D working solution 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> HBSS 	<ul style="list-style-type: none"> Wash the constructs with HBSS 2x 15 min at 37°C
6	Imaging	<ul style="list-style-type: none"> Fluorescent Microscope 	<ul style="list-style-type: none"> Bring cell-laden constructs to fluorescent microscope for imaging. Capture images in the green (FITC/488) and red (TexasRed/570) channels from both sides of the construct (dermal and epidermal).
7	Image analysis	<ul style="list-style-type: none"> Image analysis software 	<ul style="list-style-type: none"> Assess the viability percentage using any software for image analysis, e.g. Image J.

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

Applications:

- ➔ Link to Videos of some applications
- ➔ photos of some applications

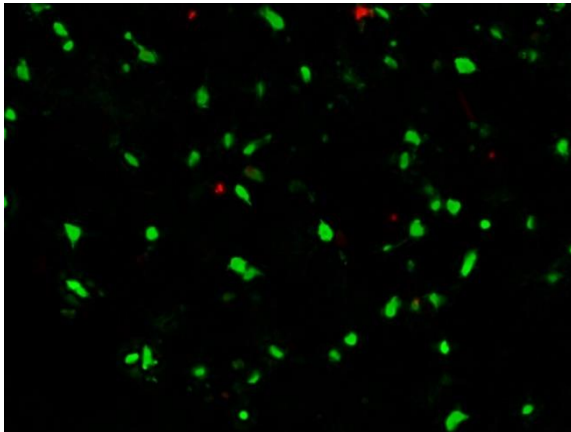


Figure 1. Epidermal side of SKIN construct day 14.

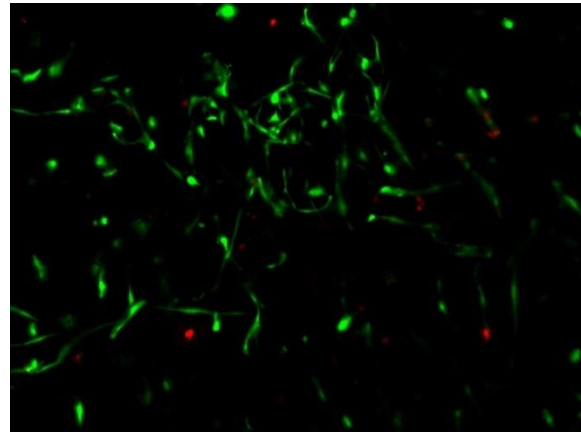


Figure 2. Dermal side of SKIN construct day 14.

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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:

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

- This protocol is optimized based on CELLINK™ Bioink, and may need further optimization for other bioinks. For more information, please contact: bioinkteam@cellink.com

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