

CELLINK FIBRIN 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
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.


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			<ul style="list-style-type: none"> ❖ 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

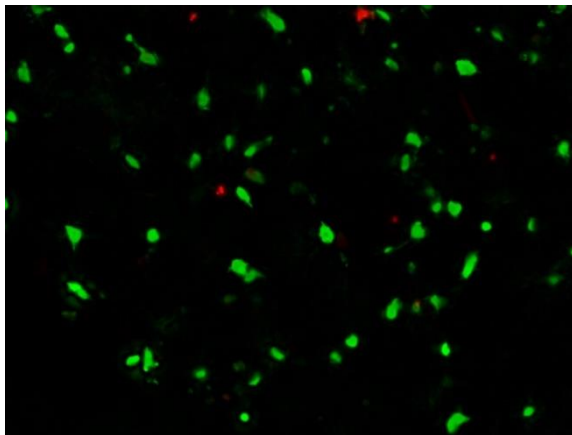


Figure 1. Epidermal side of SKIN construct day 14.

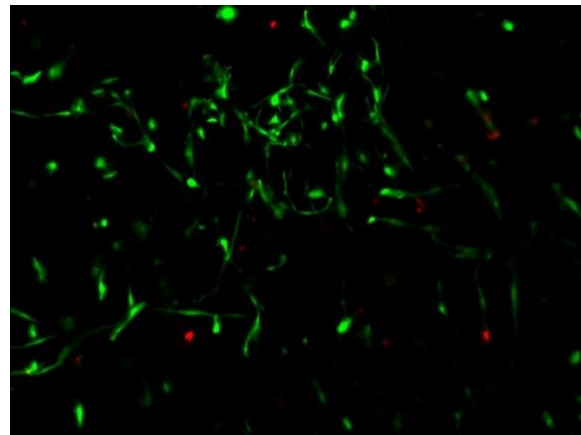



Figure 2. Dermal side of SKIN construct day 14.

 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.

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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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