

VIABILITY STAINING PROTOCOL

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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 CELLINKs bioinks.

Materials 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

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

KEEP STOCKS, WORKING SOLUTIONS AND STAINED CELLS FROM LIGHT SOURCES.

Protocol

This protocol is optimized for CELLINK bioink, GelMA and GelXA 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 center 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 such as day 7 and day 14.

1. Preparation

MATERIAL

LIVE/DEAD Cell Imaging Kit

DESCRIPTION

- Thaw LIVE/DEAD Imaging Kit Vials.

2. Washing

MATERIAL

Cell-laden printed constructs

HBSS+/-

DESCRIPTION

- Remove cell-laden constructs from the incubator. If wanted, take an extra sample to use as dead control.
- Wash constructs by carefully aspirating the medium and gently adding HBSS+/-.
- Incubate 15 min at 37°C.

3. Preparing staining solution

MATERIAL

HBSS+/-

DESCRIPTION

- 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 LIVE/DEAD working solution.

4. Apply staining solution

MATERIAL

1x LIVE/DEAD working solution

DESCRIPTION

- Remove the cell-laden constructs from the incubator.

- Aspirate the HBSS+/+ and add the 1x LIVE/DEAD working solution to the cell-laden constructs, ensuring complete liquid coverage of the constructs.
- Incubate 1h at 37°C.

5. Washing

MATERIAL

HBSS+/+

DESCRIPTION

- Wash the constructs with HBSS+/+ twice for 15 min at 37°C.

6. Imaging

MATERIAL

Fluorescent Microscope

Image analysis software

DESCRIPTION

- Bring cell-laden constructs to a fluorescent microscope for imaging.
- Capture images in the green (FITC/488) and red (TexasRed/570) channels. Capture as z-stacks or as single plane.
- Assess the viability percentage using a software for image analysis, e.g. Image J by counting the number of live and dead cells per image.

Photo of an example:

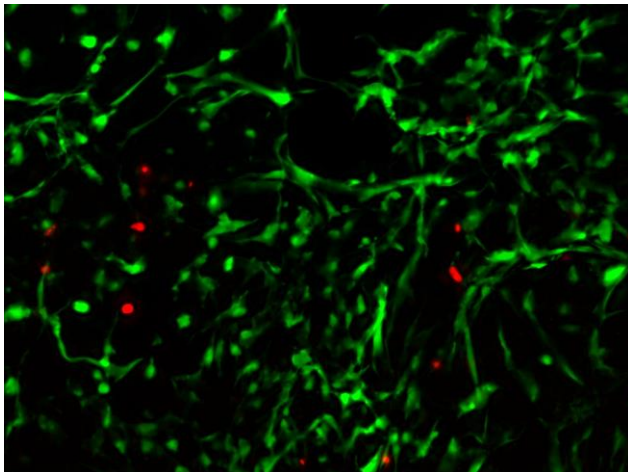


Figure 1. Human Dermal Fibroblasts in CELLINK SKIN at day 14.