

Morphology Assay

Overview: This protocol is used to assess the morphology of cells in a cell-laden 3D bioprinted construct by staining F-actin and nuclei.

Materials:

- Life Technologies ActinGreen 488 ReadyProbes (Cat No R37110)
- Life Technologies NucBlue Fixed Cells ReadyProbes (Cat No R37605)
- Fluorescent Microscope with green and blue imaging filters, set up to enable imaging
- Hank's Balanced Salt Solution (HBSS)
- Fixed cell-laden printed constructs

Protocol:

Use a benchtop shaker in step 1-6

1. Rinse fixed cell-laden constructs with HBSS at RT (see CELLINK Fixation Protocol)
2. Permeabilize constructs with 0.1 % Triton X100 for 30 min at RT
3. Rinse constructs twice: 5 min with HBSS at RT
4. Add 2 drops ActinGreen and 2 drops NucBlue to 1 ml HBSS. Add the staining solution to the cell-laden constructs, ensuring complete liquid coverage of the constructs. Cover in aluminum foil and incubate 30 min at RT.
5. Rinse constructs twice: 5 min with HBSS at RT
6. Aspirate HBSS and add an imaging solution appropriate for the microscope before capturing images in the green (FITC/488) and blue (DAPI) filters. Assess the morphology using any software for image analysis.

Further Information:

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

[ReadyProbes Ready-to-Use Imaging Reagents](#)

References:

Martínez H *et al.* 3D Bioprinting of Human Chondrocyte-laden Nanocellulose Hydrogels for Patient-specific Auricular Cartilage Regeneration. *Bioprinting*. 2016;1;22-35