

Fixation Protocol

Validated for all CELLINK bioinks, including the alginate based, nanofibrillated cellulose based, collagen based, and GelMA based bioinks. This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for fixation of cell laden constructs. Fixed samples can, among other applications, be used for multiphoton imaging or stained for immunofluorescence and immunohistology analysis.

Materials needed

- Cell laden constructs
- Formaldehyde solution (PFA) 36.5–38% (from Merck, SKU: F8775-25ML)
- Crosslinking Agent (for alginate containing bioinks)
- Hank's Balanced Salt Solution with calcium and magnesium (HBSS+/+)
- 70% Ethanol

Protocol

This protocol can be performed non-sterile, note that all handling and use of PFA must be done inside a fume hood with proper PPE and waste deposit according to local regulations.

1. Preparation of 4% PFA

MATERIAL

PFA 36.5-38%

Crosslinking Agent

DESCRIPTION

- For alginate containing bioinks, the 36.5-38% PFA can be diluted in Crosslinking Agent with 50 mM CaCl₂, to minimize the risk of the construct dissolving in the PFA. If not using alginate containing bioinks, dilute the PFA with PBS.
 - For suggested PFA, which is 36.5-38%, mix 1.1 mL of PFA with 8.9 mL PBS or 50 mM Crosslinking Agent to receive 10 mL 4% final PFA concentration.

2. Pre-wash

MATERIAL

HBSS+/-

DESCRIPTION

- Wash cell laden 3D bioprinted constructs in HBSS+/- for 10 min at 37°C.

3. Fixation

MATERIAL

4% PFA

Pre-washed, cell laden constructs

DESCRIPTION

- Submerge the 3D bioprinted samples in the 4% PFA and fix the constructs for 2-24 h at room temperature.

Note: Adjust the time according to experimental needs.

4. Washing

MATERIAL

HBSS+/-

DESCRIPTION

- Wash the constructs 3 x 10 min with HBSS+/- at room temperature.

5. Storage

MATERIAL

70% Ethanol or HBSS+/-

DESCRIPTION

- Store at 4°C in 70% ethanol or HBSS+/-.

Note: Samples can be stored in HBSS+/- at 4°C for one month. For longer storage or if proceeding to embedding, keep in 70% ethanol. For staining of the whole cell-laden 3D construct it is recommended to store the samples in HBSS+/-.