

Fixation Protocol

Validated for all CELLINK® Bioinks, including the A series, Collagen series, GelMA series, GelX series and CELLINK series. 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.

Material needed

- Cell laden constructs
- Formaldehyde solution (PFA), e.g. Merck, SKU: F8775-25ML
- 50 mM CaCl₂ solution*
- Hank's Balanced Salt Solution with calcium and magnesium (HBSS+/+)
- 70% Ethanol

*Dilute in HBSS+/+ when preparing.

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 deposit according to local regulations.

Step	Title	Material	Description
1	Preparation of 4% PFA	- PFA - 50 mM CaCl ₂	- Dilute PFA in the 50 mM CaCl ₂ solution to maintain a 4% PFA solution. - For suggested PFA, which is 36.5-38%, mix 1.1 mL of PFA with 8.9 mL 50 mM CaCl ₂ to maintain 10 mL.
2	Pre-wash	- HBSS+/-	Wash cell laden 3D bioprinted constructs in HBSS+/- for 10 min at 37°C.
3	Fixation	- 4% PFA - Pre-washed, cell laden constructs	- Fix the constructs in the 4% PFA for 2-24 h at RT. Note: Adjust the time according to experimental needs.
4	Wash	- HBSS+/-	- Wash the constructs 3 x 10 min with HBSS+/- at RT.
5	Storage	- 70% ethanol	- 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+/- . Proceed to embedding as soon as possible.