

Fixation for Cryo-sectioning Protocol

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 for cell-laden 3D bioprinted constructs for cryo-sectioning.

Material needed

- 3D bioprinted constructs
- Formaldehyde solution (PFA), e.g. Merck, SKU: F8775-25ML
- 50 mM CaCl₂ solution*
- 30% Sucrose in PBS
- Hank's Balanced Salt Solution (HBSS+/-)
- Phosphate-Buffered Saline (PBS)
- OCT Cryo-solution
- Embedding cassettes

*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	Pre-wash	- HBSS+/-	- Wash cell-laden 3D bioprinted constructs in HBSS+/- 2 x 5-10 min at 37°C.
2	Preparation of 4% PFA	- PFA - 50 mM CaCl ₂	- Dilute PFA in the 50 mM CaCl ₂ solution to obtain a 4% PFA solution. Note: For PFA with stock concentration of 36.5-38.0%, mix 1.1 ml of PFA with 8.9 ml 50 mM CaCl ₂ to obtain 10 ml of 4% PFA solution.
3	Fixation	- 4% PFA - Pre-washed, cell-laden constructs	- Fix the constructs in the 4% PFA for 2-24 hr at RT. - <i>Alternative:</i> Fix the samples 1-2 hr in RT, transfer samples to 4°C and continue the fixation for 24-48 hr. Note: Adjust the time according to experimental needs.
4	Wash	- HBSS+/-	- Wash the constructs 2 x 10 min with HBSS+/- at RT.
5	Incubation	- HBSS+/-	- Add sufficient amount of HBSS+/- to completely cover the constructs, e.g. 1 ml in a 24 well plate well. Seal the plate with parafilm and incubate at 4°C for 45 min.
6	Sucrose treatment	- 30% Sucrose in PBS	- Add sufficient amount of sucrose 30% diluted in PBS to completely cover the constructs, e.g. 1 ml in a 24 well plate well. Incubate at room temperature for 45 min.
7	Preparation of embedding cassettes	- OCT cryo-solution - Embedding cassettes	- Add OCT to the embedding cassettes and transfer the fixed and sucrose treated constructs. Ensure that the constructs are covered by OCT.
8	Storage	- -80°C freezer	- Store at -80°C until sectioning and analysis.