006005004121.GOT
Date: 09-JAN-2023
Author: IB. Version: 2

CELLINK
A BICO COMPANY

Fixation for Cryosectioning Protocol

This is a suggested procedure, please adjust according to your experimental needs. This protocol has been validated for alginate and nanofibrillated cellulose based bioinks.

Protocol aim

The aim of this protocol is to provide instructions for fixation for cell-laden 3D bioprinted constructs for cryo-sectioning.

Materials needed

- Cell laden 3D bioprinted constructs
- Formaldehyde solution (PFA) 36.5–38% from Merck, SKU: F8775-25ML
- Crosslinking Agent (for alginate containing bioinks)
- 30% Sucrose in PBS
- Hank’s Balanced Salt Solution (HBSS+/+)
- Phosphate-Buffered Saline (PBS)
- OCT
- Embedding cassettes

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 $CaCl_2$, 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 2 x 10 min in HBSS+/+ 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. Alternatively 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. Washing

MATERIAL

HBSS+/+

DESCRIPTION

- Wash the constructs 2 x 10 min with HBSS+/+ at room temperature.
- Add enough HBSS+/+ to completely cover the constructs. Seal the vessel with parafilm and incubate at 4°C for 45 min.

5. Sucrose treatment

MATERIAL

30% Sucrose in PBS

DESCRIPTION

- Add enough 30% sucrose in PBS to completely cover the constructs. Incubate at room temperature for 45 min.

6. Preparation of embedding cassettes

MATERIAL

OCT

Embedding cassettes

DESCRIPTION

- Add OCT to the embedding cassettes and transfer the fixed and sucrose treated constructs into the cassettes. Ensure that the constructs are covered by OCT.

7. Storage

MATERIAL

-80°C freezer

DESCRIPTION

- Store at -80°C until sectioning.