

## CELLINK FIBRINOGEN FIXATION FOR CRYO SECTIONING

*This is a suggested procedure, please adjust according to your experimental needs.*

### Aim of the protocol:

This protocol is used for the fixation of cells in a cell-laden 3D bioprinted construct.

### Materials needed:

- Paraformaldehyde (PFA). PFA must only be handled inside a fume hood
- 30% Sucrose in PBS
- Hank's Balanced Salt Solution (HBSS)
- OCT Cryo solution
- Embedding cassettes
- Cell-laden 3D bioprinted constructs

### Protocol:

All handling and use of PFA must be done inside a fume hood with proper PPE.

Step n°	Title	Material	Description
1	Wash	<ul style="list-style-type: none"> <li>▪ HBSS</li> <li>▪ 24 well plate</li> </ul>	❖ Transfer constructs to HBSS in a 24 well plate. 2 samples per condition.
2	Wash	<ul style="list-style-type: none"> <li>▪ HBSS</li> </ul>	❖ Wash constructs with HBSS once.
3	Fixation	<ul style="list-style-type: none"> <li>▪ 4% PFA</li> </ul>	❖ Add 1 ml 4% PFA to each well. Incubate for 1h at RT. NOTE! PFA must be handled in a fume hood
4	Wash	<ul style="list-style-type: none"> <li>▪ HBSS</li> </ul>	❖ Wash constructs with HBSS (or dPBS) twice.
5	Incubation	<ul style="list-style-type: none"> <li>▪ HBSS</li> </ul>	❖ Add 1 ml of HBSS to each well. Seal the plate with parafilm and incubate at 4°C for 45 min.
6	Sucrose treatment	<ul style="list-style-type: none"> <li>▪ 30% Sucrose in PBS</li> </ul>	❖ Add 1 ml sucrose 30% (diluted in PBS), incubate at RT for 45 min.


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7	Preparation of embedding cassettes	<ul style="list-style-type: none"> <li>▪ OCT cryo solution</li> <li>▪ Embedding cassettes</li> </ul>	❖ Add OCT to the embedding cassettes.
8	Transfer of constructs to OCT	<ul style="list-style-type: none"> <li>▪ OCT-laden embedding cassettes</li> <li>▪ Cell-laden constructs</li> </ul>	❖ Transfer constructs to OCT-laden embedding cassette (2 per cassette, on a row in the centre). Ensure that the constructs are covered by OCT.
9	Storage	<ul style="list-style-type: none"> <li>▪ -80 Freezer</li> </ul>	❖ Store at -80C until analysis.

 Want to see our talented Biologist proceed to this protocol? Feel free to find the video here:  
<https://www.youtube.com/...>

### Applications:

- ➔ Link to Videos of some applications
- ➔ photos of some applications

 Want to see our existing tissue model?  
Just go to <http://bioverse.co/> and discover a whole library of CAD files especially created for sharing 3D Bioprinting models.

- This protocol is optimized based on CELLINK™ Bioink, and may need further optimization for other bioinks. For more information, please contact: [bioinkteam@cellink.com](mailto:bioinkteam@cellink.com)

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