Application Note

Cryo-embedding with 3D CoSeedis™

Introduction:

3D CoSeedis™ is ideally suited to embed 3D spheroidal or non-spheroidal cell constructs for histological analysis such as immunohistochemistry (IHC) or immunofluorescence (IF). This application note deals with the embedding procedure to generate cryosections from 3D construct grown in 3D CoSeedis™ matrices. The underlying cell culture protocol is identical to the procedure described in our "Micro-Organoid Histology System" protocol. For further details, go to www. biopply.com.

Procedure:

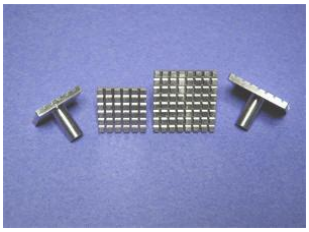
Material

- Neutral-buffered fixative (e.g. 4% formalin)
- Sucrose solutions: 10%, 20% and 30% in PBS w/o Mg^{2+}/Ca^{2+} (w/v)
- Base or plastic mould

e.g. from Fisher Scientific

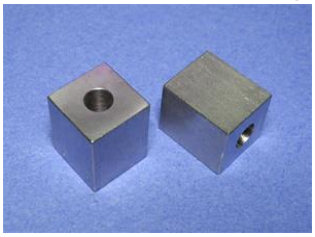
- Sample holder ("chuck";, either round or square shaped)

e.g. from Leica or



Pathology Innovations

- Over-chuck freezing blocks



e.g.from Pathology Innovations

- Embedding medium ("OCT")

Protocol

1. Remove medium from 3D CoSeedis™ matrix containing cell constructs in a multiwell plate and replace it with an appropriate volume of a neutral-buffered solution of fixative (e.g. 4% formalin). Make sure the entire matrix is covered in fixative.

2. Fix overnight at 4˚C. For prolonged fixation periods, seal plates to prevent evaporation.

3. Remove fixative from 3D CoSeedis™ matrix and replace with a solution of 10% sucrose in PBS w/o Mg^{2+}/Ca^{2+} (w/v).

4. Infiltrate overnight at 4˚C.

5. Remove the 10% sucrose solution and repeat procedure with a solution of 20% and 30% sucrose, respectively. Infiltrate overnight at 4˚C each time*).

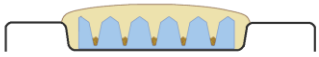
6. Cool down the over-chuck freezing blocks inside the cryostat.

7. Place fixed and sucrose-infiltrated 3D CoSeedis™ matrix onto a base or plastic mould.

7.

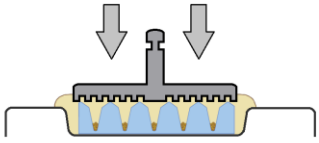
8. Cover 3D CoSeedis™ matrix with embedding medium.

8.



9. Place sample holder ("chuck") on top of 3D CoSeedis™ matrix and press down gently.

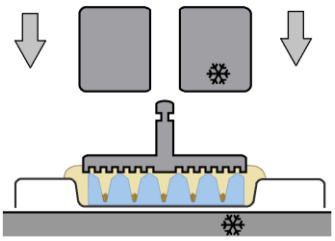
9.



*) A stepwise infiltration is optional. However, sequential increasing sucrose concentrations are considered to lead to better preservation of cell morphology.

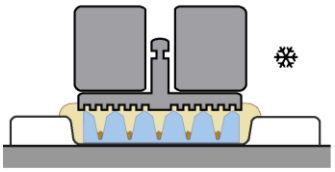
10. Place mould on cold surface (heat extractor) inside the cryostat and position over-chuck freezing blocks on top.

10.



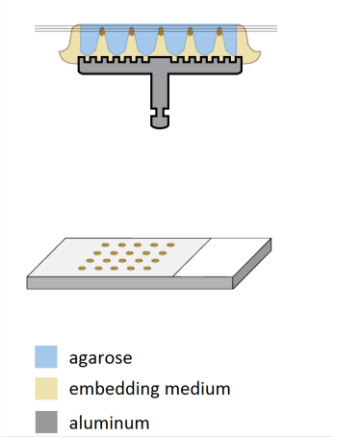
11. Allow 3D CoSeedis™ matrix to freeze completely. This will normally take approximately 2 – 3 minutes.

11.



12. Unmould 3D CoSeedis™ matrix and start with cryosections. The first cells from the 3D constructs should appear after:
    a. ~ 400 µm (1 x 1; 6-well matrices)
    b. ~ 300 µm (2 x 2; 6-well matrices)
    c. ~ 250 µm (0.5 x 0.5; 24-well matrices)

12.



Additional information:

For additional information, please contact us under service@biopply.com or contact your local abc biopply partner.
Order information can be found at:
https://biopply.com/support/order-3d-coseedis/