Embedding Protocol

**GelX Series**

Valuated for GelXA, GelXA SKIN and GelXA LAMININKS. This is a suggested procedure, please adjust according to your experimental needs.

**Protocol aim**
The aim of this protocol is to provide instructions for embedding of fixed, cell laden constructs bioprinted with CELLINK’s GelX Series which includes GelXA, GelXG, GelXA SKIN, GelXA BONE, GelXA FIBRIN and GelXA LAMININK. Embedded samples can, among other applications, be stained for immunofluorescence and immunohistology analysis.

**Material needed**
- Cell laden construct fixed according to Fixation Protocol GelX series.
- 70% ethanol (optional)*
- 96% ethanol*
- 100% ethanol*
- Xylene or xylene substitute, e.g. Shandon Xylene Substitute (Thermofisher, Ref: 9990505) *
- Paraffin
- Glass beakers of suitable size for the numbers of constructs that are to be embedded, recommended size is 250-500ml.*
- Dry oven set at 58°C*
- Embedding cassettes
- Tissue embedding machine

*Only needed for dehydration and paraffin infiltration, fixed 3D constructs can be processed with a regular tissue dehydration and infiltration machine if that is accessible.

**Protocol**
This protocol can be performed non-sterile, note that all handling and use of ethanol and xylene/xylene substitute must be done inside a fume hood with proper PPE and disposed according to local regulations.
<table>
<thead>
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<th>Step</th>
<th>Title</th>
<th>Material</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Preparation of constructs</td>
<td>- Fixed, cell laden constructs - Embedding cassettes</td>
<td>- Put fixed cell constructs in embedding cassettes and label the cassette properly with a pencil.</td>
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<tr>
<td>2</td>
<td>Preparation of paraffin</td>
<td>- Paraffin - Dry oven at 58°C</td>
<td>Note: if dehydration and paraffin infiltration is done with a tissue dehydration and infiltration machine proceed to step 5.</td>
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<td>- Fill ¾ of a suitable container with paraffin and put in the 58°C dry oven to melt.</td>
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<td>Note: This may take several hours to melt. Do not increase the temperature of the oven, higher temperatures will make the paraffin hard and brittle.</td>
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<tr>
<td>3</td>
<td>Dehydration</td>
<td>- Embedding cassettes with constructs - 70% ethanol (optional) - 96% ethanol - 100% ethanol - Xylene or xylene substitute - Glass beakers</td>
<td>Follow following dehydration series either through 1) moving the cassettes with constructs between beakers with the different reagents or 2) by adding and removing the different reagents of the dehydration series to a beaker with the cassettes.</td>
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|      |                            |                                               | 1. 70% ethanol (optional): 30 min  
2. 96% ethanol: 30 min  
3. 100% ethanol: 2 x 30 min  
4. Xylene or xylene substitute: 2 x 30 min |
|      |                            |                                               | Note: if fixed samples have been stored in 70% ethanol before embedding step 1 is not necessary.                                               |
| 4    | Paraffin infiltration      | - Paraffin at 58°C                            | - Transfer cassettes with constructs to the beakers with melted paraffin. Let sit in the oven for 60-120 min. Stir the beaker in the middle of the incubation. |
|      |                            |                                               | Note: the transfer of the cassettes to the melted paraffin must be done quickly since the paraffin solidifies <56°C.                         |
|      |                            |                                               | Note: After paraffin infiltration it is recommended to proceed to embedding as soon as possible. The infiltrated constructs can however be stored for some days at room temperature before embedding. |
| 5 | Paraffin embedding | - Let the excess paraffin melt away from the cassettes with constructs by leaving them in the cassette holder pocket in the tissue embedding machine for ~20 min.
- Open the cassette. Fill a metallic embedding mould with paraffin, with tweezers, push the construct (crossection down) to the bottom of the mould and let stiffen through cooling. Add the bottom of the embedding cassette on the top of the mould before the paraffin stiffens. Throw the lid.
- Leave the embedded construct at the cooling plate until it easily can be removed from the mould (~20 min).

Note: Don’t leave the mould with the embedded construct at the cooling plate for too long, if the paraffin gets too cold it can break. | Tissue embedding machine |