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Embedding of Tissues in Paraffin

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol aim

The aim of this protocol is to provide instructions for paraffin embedding of fixed, cell laden constructs. Embedded samples can, among other applications, be stained for immunofluorescence and immunohistology analysis. Follow Fixation Protocol before starting this protocol.

Materials Needed

- Paraffin
- Dry oven at 58°C
- Fixed, cell laden construct
- Embedding cassettes
- 70% Ethanol
- 96% Ethanol
- 100% Ethanol
- Xylene or xylene substitute, e.g. Shandon Xylene Substitute (Thermofisher, Ref: 9990505)
- Tissue embedding machine

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. Dispose waste according to local regulations. If performing dehydration and paraffin infiltration with tissue dehydration and infiltration machine, it’s recommended to test the automated process with spare samples before using with sensitive samples. If dehydration and paraffin infiltration is done with a tissue dehydration and infiltration machine only step 2 and 5 is performed in the protocol.

Paraffin Embedding

1 **Preparation of paraffin**

- Paraffin
- Dry oven at 58°C

Fill ¾ of a suitable container with paraffin and put in the 58°C dry oven to melt.

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.

2 **Preparation of constructs**

Fixed, cell laden tissues

Embedding casettes

Put fixed, cell laden constructs in embedding cassettes and label the cassette properly with a pencil.

3 **Dehydration**

- Embedding casettes with tissues
- 70% ethanol
- 96% ethanol
- 100% ethanol
- Xylene or xylene substitute
- Glass breakers

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.

Handle both xylene and ethanol with care inside a fume hood with proper PPE.

1. 70% ethanol: 2 x 30 min
2. 96% ethanol: 2 x 30 min
3. 100% ethanol: 2 x 30 min
4. Xylene or xylene substitute: 3 x 30 min

Note 1: If fixed samples have been stored in 70% ethanol before embedding only 1 x 30 min of 70% is necessary.

Note 2: The constructs might shrink after the dehydration procedure depending on the bioink type.

4 **Paraffin infiltration**

- Paraffin at 58°C

Transfer cassettes with constructs to the beaker with melted paraffin. Let sit in the oven for 60 min (x2).

Transfer cassettes to final paraffin solution and leave overnight.

Note 1: The transfer of the cassettes to the melted paraffin must be done quickly since the paraffin solidifies under 56°C.

5 **Paraffin embedding**

- Tissue embedding machine

Add the infiltrated samples to the cassette container of the tissue embedding machine.

To embed samples:

- Open the cassette.
- Fill a metallic embedding mould with paraffin.
- With warm tweezers; transfer the constructs. from the cassette to the mould with paraffin and push the constructs (cross-section down) to the bottom of the mould.
- Let stiffen slightly on a cold plate so the sample stays at the bottom of the mould.
- Add the lid of the embedding cassette on the top of the mould before the paraffin stiffens completely. - - Throw the bottom.
- Leave the embedded construct at the cooling plate until it easily can be removed from the mould (~20 min).

Note 1: 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.

Note 2: The paraffin embedded blocks can be stored at room temperature for an extended period of time.