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Paraffin Embedding Protocol

Validated for all CELLINK bioinks, including the alginate based, nanofibrillated cellulose based, collagen based, and GelMA based bioinks. This is a suggested procedure, please adjust according to your experimental needs.

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.

1. Preparation of paraffin

MATERIAL

Paraffin

Dry oven at 58°C

DESCRIPTION

- 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

MATERIAL

Fixed, cell laden constructs

Embedding cassettes

DESCRIPTION

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

3. Dehydration

MATERIAL

Embedding cassettes with constructs

70% ethanol

96% ethanol

100% ethanol

Xylene or xylene substitute

DESCRIPTION

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

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

4. Paraffin infiltration

MATERIAL

Paraffin at 58°C

DESCRIPTION

- Transfer cassettes with constructs to the beaker with melted paraffin. Let sit in the oven for 45 min.

Note: The transfer of the cassettes to the melted paraffin must be done quickly since the paraffin solidifies under 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

MATERIAL

Tissue embedding machine

DESCRIPTION

- Add the infiltrated samples to the cassette container of the tissue embedding machine.
- If samples have been stored in room temperature before embedding let the excess paraffin melt away from the cassettes by leaving them in the cassette holder pocket for ~20 min. If proceeding directly from infiltration, step 4, you can start embedding after a few minutes since the paraffin is already warm.
- To embed samples:
    1. Open the cassette.
    2. Fill a metallic embedding mould with paraffin.
    3. With warm tweezers; transfer the constructs. from the cassette to the mould with paraffin and push the constructs to the bottom of the mould. If applicable, place the cross-section down.
    4. Let stiffen slightly on a cold plate so the sample stays at the bottom of the mould.
    5. Add the lid of the embedding cassette on the top of the mould before the paraffin stiffens completely. Throw the bottom.
    6. 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.