006005004136.GOT
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Author: IB,JH. Version: 2

Sectioning Protocol

Validated for all CELLINK bioinks, including alginate, nanofibrillated cellulose, collagen 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 sectioning of paraffin embedded constructs. Embedded samples can, among other applications, be stained for immunofluorescence and immunohistology analysis. Follow *Fixation Protocol* and *Paraffin Embedding Protocol* before starting this protocol.

Materials needed

- Embedded constructs
- Rotary microtome
- Water bath at ~39°C
- Pencils/brushes
- Microscope slides, e.g. VWR Microscope slides Ref:63-1163
- Dry oven at 56°C

Protocol

The blade needs to be handled with care. Always put back the safety block when leaving the machine and always lock the rotary wheel when not in use or when changing construct.

1. Preparation of samples

MATERIAL

20°C Freezer

DESCRIPTION

- Prepare the embedded samples for sectioning by putting them in a -20°C freezer for ~20 min.

Note: The paraffin can become brittle and break if the samples are left in the freezer for too long. Do not leave in the freezer longer than needed or at colder temperatures.

2. Mounting on microtome

MATERIAL

Embedded construct

Rotary microtome

DESCRIPTION

- Attach the embedded construct to the microtome by putting it in the rectangular space, construct facing blade.
- Make sure that the embedded construct is in vertical line with the blade for the best cuts.

3. Sectioning

MATERIAL

Embedded construct in rotary microtome

DESCRIPTION

- Adjust the thickness of the slides, start at 30 µm or 20 µm, decrease to 10 µm when at the embedded construct and decrease to 5 µm when you are in the construct.

Note: Adjust slide thickness according to experimental needs.

4. Attaching to slides

MATERIAL

Water bath at ~39°C

Pencils/brushes

Microscope slides

DESCRIPTION

- Transfer the 5 µm sections to the surface of the water bath with pencils or brushes. Let stretch out, make sure the section not folds when dropped onto the water surface.

- Collect the sections on the treated side of a microscope slide.

Note: If the sections do not stretch out when dropped on the surface of the water the water is too cold. If the slides dissolve the water is too warm.

5. Drying

MATERIAL

Dry oven at 56°C

DESCRIPTION

- Secure the attachment of the sections to the microscope slide by incubating them at 56°C for 20 min.
- Let the slides air dry overnight in room temperature.