

Sectioning Protocol

CELLINK Series

Validated for CELLINK® Bioink, CELLINK® RGD, CELLINK® FIBRIN, CELLINK® LAMININKS and CELLINK® SKIN. 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 bioprinted with CELLINK® Bioink Series which includes CELLINK® Bioink, CELLINK® BONE, CELLINK® FIBRIN, CELLINK® LAMININK, CELLINK® RGD and CELLINK® SKIN. Embedded samples can, among other applications, be stained for immunofluorescence and immunohistology analysis.

Material needed

- Embedded construct embedded according to *Embedding Protocol CELLINK Series*.
- Rotary microtome
- Water bath at ~40°C
- Pencils
- Microscope slides, e.g. VWR Microscope slides Ref:63-1163
- Dry oven at 56°C

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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 change construct.

Step	Title	Material	Description
1	Prepare samples	- -20°C	- 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	Mount microtome	- Rotary Microtome - Embedded construct	- Attach the embedded construct to the microtome by putting it in the rectangular space, construct facing blade.
3	Sectioning		- By moving either the holder of the construct (the rotary wheel on the left side of the microtome) or the blade make sure the face of the embedded construct is in vertical line with the blade. - 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: The 5 μm sections are what you want in this experiment, adjust according to experimental needs.
4	Attach to slides	- Water bath - Pencils - Microscope slides	- Transfer the 5 μm sections to the surface of the water bath with pencils. Let stretch out, make sure the section not folds when dropped onto the water. - 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 hot.
5	Drying	- Dry oven	- Secure the attachment of the sections to the microscope slide by keeping them at 56°C for 20 min. - Let the slides air dry overnight in room temperature.