

H&E Staining 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 H&E staining of sectioned paraffin embedded constructs bioprinted with CELLINK® Bioink Series which includes CELLINK® Bioink, CELLINK® BONE, CELLINK® FIBRIN, CELLINK® LAMININK, CELLINK® RGD and CELLINK® SKIN.

Material needed

- Microscope slides with sectioned construct sectioned according to *Sectioning Protocol CELLINK Series*.
- Beakers for microscope slides
- Distilled water
- 96% Ethanol
- 100% Ethanol
- Xylene or xylene substitute, e.g. Shandon Xylene Substitute (Thermofisher, Ref: 9990505)
- H&E staining kit, Vector Laboratories Cat.No.:H-3502
- Cover glass
- Mounting medium, e.g. Vector Laboratories Cat.No.:H-5000

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Protocol

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 regulation.

Step	Title	Material	Description
1	Deparaffination and re-hydration	<ul style="list-style-type: none"> - Microscope slides with sectioned construct - Distilled water - 96% Ethanol - 100% Ethanol - Xylene or xylene substitute 	<p>Deparaffinize and rehydrate sections by moving microscope slides with sectioned construct through following series:</p> <ol style="list-style-type: none"> 1. Xylene or xylene substitute: 3 x 3 min 2. 100% ethanol: 1 min 3. 96% ethanol: 1 min 4. Distilled water, at least 2 min
2	H&E stain	<ul style="list-style-type: none"> - H&E staining kit 	<p>Apply the staining solutions in following order, always adding enough to completely cover the sections.</p> <ol style="list-style-type: none"> 1. Haematoxylin, 3 min 2. Rinse in 2 changes of distilled water, 15 sec each 3. Bluing agent, 10-15 seconds 4. Rinse in 2 changes of distilled water, 15 sec each 5. Dip slide in 100% ethanol 10 seconds 6. Eosin Y Solution, 4 min 7. Rinse slide in 100% ethanol 10 seconds <p>Note: Blot off excess solution between the steps through tapping the slide (on the side, i.e. so it is held vertically) towards a paper covered area of the bench. Especially important after the washes since excess water/ethanol will dilute the staining solutions if not removed.</p>
3	Dehydration and clearing	<ul style="list-style-type: none"> - 100% Ethanol - Xylene or xylene substitute 	<p>Dehydrate and clear slides by moving the stained microscope slides through following series:</p> <ol style="list-style-type: none"> 1. 100% ethanol: 3 x 1 min 2. Xylene or xylene substitute: 3 x 1 min
4	Mount and coverslip	<ul style="list-style-type: none"> - Mounting medium - Cover glass 	<ul style="list-style-type: none"> - Apply a drop of mounting medium to the stained slides. - Cover with a cover glass, apply carefully to avoid air bubbles. - Let air dry placed horizontally overnight.