

H&E Staining Protocol

Validated for all CELLINK® Bioinks, including the A series, Collagen series, GelMA series, GelX series and CELLINK series. 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.

Material needed

- Microscope slides with sectioned construct according to *Sectioning Protocol*
- 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

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.

Step	Title	Material	Description
1	Deparaffination and rehydration	<ul style="list-style-type: none"> - Microscope slides with sectioned construct - Distilled water - 96% Ethanol - 100% Ethanol - Xylene or substitute 	<ul style="list-style-type: none"> - Deparaffinize and rehydrate sections by moving microscope slides with sectioned construct through following series: <ol style="list-style-type: none"> 1. Xylene or xylene substitute: 3 x 5 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 	<ul style="list-style-type: none"> - Apply the staining solutions in following order, always adding enough to completely cover the sections. <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 sec 4. Rinse in 2 changes of distilled water, 15 sec each 5. Dip slide in 100% ethanol 10 sec 6. Eosin Y Solution, 4 min 7. Rinse slide in 100% ethanol 10 sec - 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. - Note: If using another H&E kit, adapt accordingly.
3	Dehydration and clearing	<ul style="list-style-type: none"> - 100% Ethanol - Xylene or xylene substitute 	<ul style="list-style-type: none"> - Dehydrate and clear slides by moving the stained microscope slides through following series: <ol style="list-style-type: none"> 1. 100% ethanol: 2 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.