

H&E Staining 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 haematoxylin and eosin (H&E) staining of sectioned paraffin embedded constructs. Follow *Paraffin Embedding Protocol* and *Sectioning Protocol* before starting this protocol.

Materials needed

- Microscope slides with sectioned construct
- Beakers for microscope slides
- Xylene or xylene substitute, e.g., Shandon Xylene Substitute (ThermoFisher, Ref: 9990505)
- 100% Ethanol
- 96% Ethanol
- Distilled water
- 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.

1. Deparaffination and rehydration

MATERIAL

Microscope slides with sectioned construct

Beakers for microscope slides

Xylene or substitute

100% Ethanol

96% Ethanol

Distilled water

DESCRIPTION

- Deparaffinize and rehydrate sections by moving microscope slides with sectioned construct through the following series of liquids in beakers for microscope slides:
 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

MATERIAL

H&E staining kit

DESCRIPTION

- Blot of the distilled water and apply the staining solutions in following order, always add enough to completely cover the sections:
 1. Haematoxylin for 3 min
 2. Rinse in 2 changes of distilled water for 15 s each
 3. Bluing agent for 10-15 s
 4. Rinse in 2 changes of distilled water for 15 s each
 5. Dip slide in 100% ethanol for 10 s
 6. Eosin Y Solution for 4 min
 7. Rinse slide in 100% ethanol for 10 s

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

MATERIAL

100% Ethanol

Xylene or xylene substitute

DESCRIPTION

- Dehydrate and clear slides by moving the stained microscope slides through following series:
 1. 100% ethanol: 2 x 1 min
 2. Xylene or xylene substitute: 3 x 1 min

4. Slide preparation

MATERIAL

Mounting medium

Cover glass

DESCRIPTION

- Blot of the excess solution and 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.