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Trichrome Staining 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 trichrome staining of sectioned paraffin embedded constructs. Follow *Fixation Protocol*, *Paraffin Embedding Protocol and Sectioning Protocol* before starting this protocol.

Materials needed

- Microscope slides with sectioned construct
- Recommended thickness: 8-12 µm
- Beakers for microscope slides
- Distilled water
- 96% Ethanol
- 100% Ethanol
- Xylene or xylene substitute, e.g. Shandon Xylene Substitute (Thermofisher, Ref: 9990505)
- Microscope slide box
- 56°C oven
- Trichrome staining kit, Abcam Cat. No.: ab150686
- Mounting medium, e.g. Vector Laboratories Cat.No.:H-5000
- Cover glass

Protocol

All handling and use of dyes, ethanol and xylene/xylene substitute must be done inside a fume hood with proper PPE and disposed according to local regulation. If using another Trichrome Staining kit adjust the protocol accordingly.

1. Deparaffination and rehydration

MATERIAL

Microscope slides with sectioned construct

Xylene or xylene substitute

100% Ethanol

96% Ethanol

Distilled water

DESCRIPTION

- Deparaffinize and rehydrate sections by moving microscope slides with sectioned construct through following series:
    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. Trichrome stain, Bouin's Fluid

MATERIAL

Microscope slide box

Bouin's Fluid

Oven at 56°C

DESCRIPTION

- Make a humified chamber of the microscope box by adding wet paper at the bottom.
- Blot of the samples and place the microscope slides horizontally in the humified chamber.
- Add Bouin's Fluid to the sections, close the box and carefully transfer the box to the oven.
- Incubate 60 min followed by a 10 min cooling period.
- Rinse in tap water until sections are completely clear.
- Rinse once in distilled water.

Note: Bouin's Fluid is toxic, keep lid properly closed whenever not within a fume hood and handle with outermost care.

3. Trichrome stain, continued

MATERIAL

Trichrome staining kit

96% Ethanol

DESCRIPTION

- Apply the staining solutions in following order, always adding enough to completely cover the sections:
    1. Mix equal amounts of Weigert's (A) and Weigert's (B) to maintain working solution of Weigert's Iron Haematoxylin: 5 min.
    2. Rinse in tap water until sections are completely clear.
    3. Rinse once in distilled water.
    4. Biebrich Scarlet/Acid Fuchsin Solution: 15 min.
    5. Rinse in distilled water.
    6. Phosphomolybdic/Phosphotungstic Acid: 15 min.
    7. Remove Phosphomolybdic/Phosphotungstic Acid but do not rinse.
    8. Aniline Blue solution: 7 min.
    9. Rinse in distilled water.
    10. Acetic acid solution (1%): 3-5 min.
    11. Dehydrate slides quickly in two changes of 96% ethanol.

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.

4. 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

5. Mount and coverslip

MATERIAL

Mounting medium

Cover glass

DESCRIPTION

- Apply a drop of mounting medium to the stained slides.
- Cover with a cover glass, apply carefully to avoid air bubbles.
- Let air dry horizontally overnight.