Trichrome 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 trichrome staining of sectioned paraffin embedded constructs.

Material needed

- Microscope slides with sectioned construct sectioned according to *Sectioning Protocol*
  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
- Cover glass
- Mounting medium, e.g. Vector Laboratories Cat.No.:H-5000

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.

Boston, USA
75 Kneeland Street
Boston, MA 02111

Gothenburg, Sweden
Arvid Wallgrens Backe 20,
Gothenburg, 41346

Blacksburg, USA
2000 Kraft Dr, Suite 2125
Blacksburg, VA 24060

Kyoto, Japan
46-29 Yoshida-Shimo Adachi-cho, Sakyo-ku, Kyoto

www.cellink.com | +1 (833) CELLINK

Step	Title	Material	Description
1	Deparaffination and re-hydration	- Microscope slides with sectioned construct - Distilled water - 96% ethanol - 100% ethanol - Xylene or xylene substitute	- 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	- Microscope slide box - Bouin's Fluid - Oven at 56°C	- 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.

Boston, USA
75 Kneeland Street
Boston, MA 02111

Gothenburg, Sweden
Arvid Wallgrens Backe 20,
Gothenburg, 41346

Blacksburg, USA
2000 Kraft Dr, Suite 2125
Blacksburg, VA 24060

Kyoto, Japan
46-29 Yoshida-Shimo Adachi-cho, Sakyo-ku, Kyoto

www.cellink.com | +1 (833) CELLINK

3	Trichrome stain, continued	- Trichrome staining kit - 96% Ethanol	1. Apply the staining solutions in following order, always adding enough to completely cover the sections. 2. Mix equal amounts of Weigert's (A) and Weigert's (B) to maintain working solution of Weigert's Iron Haematoxylin, 5 min. 3. Rinse in tap water until sections are completely clear. 4. Rinse once in distilled water. 5. Biebrich Scarlet/Acid Fuchsin Solution, 15 min. 6. Rinse in distilled water. 7. Phosphomolybdic/Phosphotungstic Acid, 15 min. Note, remove but do not rinse before adding Aniline Blue solution. 8. Aniline Blue solution, 7 min. 9. Rinse in distilled water. 10. Acetic acid solution (1%), 3-5 min. 11. Dehydrate slides quickly in 2 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	- Ethanol - Xylene or xylene substitute	- 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	- Mounting medium - Cover glass	- 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.

Boston, USA
75 Kneeland Street
Boston, MA 02111

Gothenburg, Sweden
Arvid Wallgrens Backe 20,
Gothenburg, 41346

Blacksburg, USA
2000 Kraft Dr, Suite 2125
Blacksburg, VA 24060

Kyoto, Japan
46-29 Yoshida-Shimo Adachi-cho, Sakyo-ku, Kyoto

www.cellink.com | +1 (833) CELLINK