Sectioned Construct

Immunofluorescence 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 immunofluorescence staining of paraffin embedded, sectioned 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)
- PBS/TBS
- Triton 100X or Tween20
- Antigen retrieval buffer (Tri-sodium citrate, distilled water, sodium hydroxide/acetic acid, Triton 100X/Tween20)
- Water bath that can reach 98-100°C
- Super PAP pen (optional)
- Bovine serum albumin (BSA) or serum of host of secondary antibody
- Microscope slide box
- Primary antibody
- Secondary antibody
- DAPI (1 mg/mL)
- Cover glass
- Mounting medium, e.g. Fluoromount G

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.

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Step	Title	Material	Description
1	Prepare antigen retrieval buffer	- Tri-sodium citrate - Distilled water - sodium hydroxide/acetic acid - Triton 100X/Tween20	Note: If antigen retrieval buffer already is prepared, proceed to step 2. - Prepare antigen retrieval buffer by: 1. Dilute 2.94 g of dihydrate tri-sodium citrate in 1000 mL of distilled water 2. Adjust the pH to 6.0 with sodium hydroxide/acetic acid 3. Add 0.5 mL of Tween 20/Triton 100x Antigen retrieval buffer can be stored 1 month in RT, for extended storage store at 4°C.
2	Prepare blocking solution	- BSA or serum of host of secondary antibody - TBS/PBS - Triton 100X/Tween20	Note: If blocking solution already is prepared, proceed to step 3. Prepare blocking solution by mixing 3% BSA or serum of host of secondary antibody with PBST/TBST (PBS or TBS with 0.05% Triton 100X or Tween20). Store at 4°C.
3	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
4	Antigen retrieval	- Antigen retrieval buffer - Water bath - Slide container that stays intact at 100°C, e.g. a plastic container	- Put the microscope slides in the container and fill up with antigen retrieval buffer until the slides are covered. - Place the container with samples and retrieval buffer in the water bath. - Turn on heating and let the water reach boiling (98°C-100°C). Let the samples incubate 10 min, starting from when the water starts boiling (above 98°C ok). - Let the samples cool in the buffer. Note: To speed up cooling put the container with samples and retrieval buffer in cold water after treatment.

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5	Preparation of samples	- TBS/PBS - Super PAP pen (optional)	- Rinse slides 2 x 3 min in PBS or TBS - Let slides dry and circle the sections with the Super PAP pen to separate/highlight them. This allows for multiple staining on slides.
6	Blocking	- Blocking solution	- Add blocking solution to the sections, incubate 45 min in RT. - Blot of blocking solution, do not rinse. Note: Always add enough solution to completely cover the sections.
7	Primary antibody	- Microscope slide box - Primary antibody - Blocking solution - PBS/TBS	- Make a humified chamber of the microscope box by adding wet paper at the bottom of the box. - Place slides horizontally in the humified chamber. - Dilute primary antibody in blocking solution at recommended concertation, calculate 40-50 µL per section. - Add enough primary antibody to completely cover the sections. - Place horizontally in humified chamber over night at 4°C or in RT 60 min. - Rinse 2 x 3 min with PBS or TBS.
8	Secondary antibody	- Microscope slide box - Secondary antibody - Blocking solution	Note: Make sure to work without UV in darkness to not bleach the fluorophores. - Blot off excess PBS/TBS, important to not dilute secondary antibody. - Dilute secondary antibody in blocking solution at recommended concertation, calculate 40-50 µl per section. - Add enough secondary antibody to completely cover the sections. - Incubate 60 min in humified chamber at RT.
9	DAPI stain	- DAPI (1 mg/mL) - PBS/TBS	Note: Make sure to work without UV in darkness to not bleach the fluorophores. - Dilute DAPI (1 mg/mL) 1:50 in PBS or TBS. - Blot off secondary antibody and add enough DAPI to completely cover the sections. - Incubate 10 min in RT in humified chamber. - Rinse 2 x 3 min with PBS or TBS.
10	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 dry at 4°C horizontally overnight.

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