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Immunofluorescence 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 immunofluorescence staining of paraffin embedded, sectioned constructs. Follow *Paraffin Embedding Protocol* and *Sectioning Protocol* before starting this protocol.

Materials needed

- Antigen retrieval buffer (Tri-sodium citrate, distilled water, sodium hydroxide/acetic acid, Triton 100X/Tween20)
- Distilled water
- Bovine serum albumin (BSA) or serum of host of secondary antibody
- PBS/TBS
- Triton 100X or Tween20
- Microscope slides with sectioned construct
- 96% Ethanol
- 100% Ethanol
- Xylene or xylene substitute, e.g. Shandon Xylene Substitute (ThermoFisher, Ref: 9990505)
- Water bath that can reach 98-100°C
- Microscope slide container
- Super PAP pen
- 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 waste disposed according to local regulation.

1. Preparing antigen retrieval buffer

MATERIAL

Dihydrate tri-sodium citrate

Distilled water

Sodium hydroxide/acetic acid

Triton 100X/Tween20

DESCRIPTION

Note: If antigen retrieval buffer already is prepared, proceed to Step 2.

- Prepare antigen retrieval buffer by:
    1. Diluting 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.
    4. Mix the solution properly.
- Antigen retrieval buffer can be stored 1 month in room temperature, for extended storage store at 4°C.

2. Preparing blocking solution

MATERIAL

BSA or serum of host of secondary antibody

PBS/TBS

Triton 100X/Tween20

DESCRIPTION

Note: If blocking solution already is prepared, proceed to Step 3.

- Prepare a 3% blocking solution by dissolving 1.5 g of BSA or serum of host of secondary antibody in 50 mL PBS or TBS. Add 50 µL Triton 100X /Tween20 for a 0.1% final concentration. Store at 4°C.

3. Deparaffination and re-hydration

MATERIAL

Microscope slides with sectioned construct

Xylene or xylene substitute

100% ethanol

Distilled water

96% ethanol

DESCRIPTION

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

MATERIAL

Antigen retrieval buffer

Water bath

Microscope slide container (stays intact at 100°C)

DESCRIPTION

- 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 cold 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.

5. Preparation of samples

MATERIAL

PBS/TBS

Super PAP pen

DESCRIPTION

- Rinse slides 2 x 3 min in PBS/TBS
- Let slides dry and circle the sections with the Super PAP pen to separate/highlight them. This allows for multiple staining on the same slide.

6. Blocking/permeabilization

MATERIAL

Blocking solution

DESCRIPTION

- Add blocking solution to the sections, incubate 45 min at room temperature.
- Blot of blocking solution, do not rinse.

Note: Always add enough solution to completely cover the sections.

7. Primary antibody

MATERIAL

Microscope slide box

Primary antibody

Blocking solution

PBS/TBS

DESCRIPTION

- 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 concentration, 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 room temperature for 60 min.
- Rinse 2 x 3 min with PBS/TBS.

8. Secondary antibody

MATERIAL

Microscope slide box

Secondary antibody

Blocking solution

DESCRIPTION

- Make sure to work in darkness to not bleach the fluorophores.
- Blot off excess PBS/TBS, it's important to not dilute secondary antibody.
- Dilute the 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 room temperature.

9. DAPI stain

MATERIAL

DAPI (1 mg/mL)

PBS/TBS

DESCRIPTION

- Make sure to work in darkness to not bleach the fluorophores.
- Dilute DAPI (1 mg/mL) 1:50 in PBS/TBS.
- Blot off secondary antibody and add enough DAPI to completely cover the sections.
- Incubate 10 min in room temperature in humified chamber.
- Rinse 2 x 3 min with PBS/TBS.

10. Prepare slides

MATERIAL

Mounting medium

Cover glass

DESCRIPTION

- Blot of the PBS/TBS and 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.