SEP 08, 2023

OPEN ACCESS

Protocol Citation: Gabriela Vallejo Flores, Annika Fendler 2023. Immunofluorescence staining for tissue and organoids . **protocols.io** https://protocols.io/view/immunofluorescence-staining-for-tissue-and-organoi-cy35xyq6

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
We are still developing and optimizing this protocol

Created: Aug 23, 2023

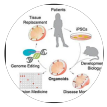
Last Modified: Sep 08, 2023

PROTOCOL integer ID: 86877

Immunofluorescence staining for tissue and organoids

Gabriela Vallejo Flores[1], Annika Fendler[1]

[1]Charité



Gabriela Vallejo Flores

ABSTRACT

Immunohistochemistry (IHC) is a powerful technique that exploits the specific binding between an antibody and antigen to detect and localize specific antigens in cells and tissue, most commonly detected and examined with the light microscope.

This protocol will be used to analyze the presence of some protein in Bladder tissue and organoids tumor and non-tumor, some of the antigens are the following CK20, CK14, CK5, CTK18.

GUIDELINES

Prepare the solutions needed for this protocol one or two days before starting the sample processing:

- TBS 1x
- TBS 1x 0.05% of Tween
- Buffer A: TBS with 1% of BSA + 2% of host serum of the secondary antibody
- Buffer B: with 1% of BSA
- Cuts the slides some days before the day of the staining

MATERIALS

- Primary antibody
- Secondary antibody
- ProLong Gold with DAPI **Contributed by users Catalog #P36931**
- Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9418**
- Normal Donkey Serum **Jackson ImmunoResearch Laboratories, Inc. Catalog #017-000-121**
- TBS 1x
- Tween 20
- Pan pen
- Humidifying box
- Glass staining dish with cover
- Stainless steel rack

BEFORE START INSTRUCTIONS

- Before starting the protocol, place the primary antibodies on ice.
- Label the tubes for the primary antibodies dilutions
- During the incubation time of the blocking buffer, dilute the primary antibodies in blocking buffer B
- Prepare the humidifying box
- use the pan pen to delimit the sample

Antibodies in use

1

	Date	Sample ID	Antibody	Host	Datasheet dilution	Cat No.	Lot

	Date	Sample ID	Antibody	Host	Datasheet dilution	Cat No.	Lot

Table 1. Description of the antibodies

2 Delimit the sample with a pan pen, add TBS 1x to the sample and check that the buffer is not licking.

Blocking

3 Add blocking buffer A (1% BSA + 2% of host serum (secondary antibody) in TBS 1x, add 150µl for tissue and 50µl for organoids staining, incubate the sample 60 min a room temperature in the humidifying box.

4 Remove the blocking buffer A with a towel in the corners around the sample.

Primary antibody

5 Add the primary antibody to the sample, dilute the antibody in blocking buffer B (1% BSA in TBS 1x) and Add 150µl for tissue and 50µl for organoids staining, incubate the sample a room temperature or overnight at 4°C in a humidifying box.

	Sample ID	Sample No.	Antibody 1	Antibody 2	Host 1	Host 2	Dilutions Ab1: Ab2

	Sample ID	Sample No.	Antibody 1	Antibody 2	Host 1	Host 2	Dilutions Ab1: Ab2

Table 2. Description of primary antibodies

6 Remove the primary antibody with a towel in the corners around the sample.

7 Wash 3 times the sample with TBS 1X; 0.05% tween 20 and one time with TBS 1X.

Secondary antibody

8 Add the secondary antibody to the sample, dilute the antibody in blocking buffer B, and add Hoesch 1:200 for nuclea staining, and incubate the sample 30 min a room temperature in a humidifying box.

	Sample ID	Sample No.	Antibody 1	Antibody 2	Dilution 1	Dilution 2

Table 3.Description of secondary antibodies

9 Remove the secondary antibody with a towel in the corners around the sample.

10 Wash 4 times the samples with 0.05% tween in TBS 1x.

11 Wash one time the samples with TBS 1x.

Sample Mounting

12 Add 2 drop of antifade solution(a mounting medium that Inhibits photobleaching of fluorescent dyes and fluorescent proteins, No warming necessary, Can be stored without sealing for long term analysis)

13 Let dry the slides for 10 min a room temperature, then are ready for us.

14 The slide can be keeping at 4°C for 1 or 2 weeks or minus 20 for some months.

Results

15

Note

16

Note

17

18

19

20