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Immunofluorescence staining of paraffin embedded cell block sections

Oncoimmunology

Erez Nissim Baruch[1], Rona Ortenberg[2]

[1]Department of Clinical Immunology and Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Israel,The Ella Lemelbaum Institute for Immuno-Oncology, Sheba Medical Center, Tel-HaShomer, Israel,

[2]The Ella Lemelbaum Institute for Immuno-Oncology, Sheba Medical Center, Tel-HaShomer, Israel

1 Works for me dx.doi.org/10.17504/protocols.io.6pqhdmw

Rona Ortenberg

ABSTRACT

This protocol is designated for Immunofluorescence staining of paraffin embedded cell block sections.

EXTERNAL LINK

https://doi.org/10.1080/2162402X.2020.1741267

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Baruch EN, Ortenberg R, Avivi C, Anafi L, Dick-Necula D, Stossel C, Moshkovits Y, Itzhaki O, Besser MJ, Schachter J, Barshack I, Markel G, Immune co-culture cell microarray – a feasible tool for high-throughput functional investigation of lymphocyte–cancer interactions. Oncoimmunology 9(1). doi: 10.1080/2162402X.2020.1741267

MATERIALS

NAME	CATALOG #	VENDOR
Ethanol 100%		
Tween 20	P1379-500ml	Sigma-aldrich
Xylene	XC9800.SIZE.1L	Bio Basic Inc.
Fluoromount	F4680	Sigma
DAPI	D3571	Invitrogen - Thermo Fisher
DPBS no calcium no magnesium	02-023-1A	
10x Citrate Buffer pH6.0	KO35	

1 1. After the slides were cut, let them dry in room temprature over-night, or 2-3 hours on a heating plate at 37°C.
2. Before stainig incubate the slides in oven at 55°C for 1 - 2 hours

2 In a chemical hood:
1. Wash in Xylene for 10 minutes
2. Wash in new Xylene for another 10 minutes
3. Wash in Ethanol 100% for 3 minutes

3 On the bench, room temprature:
1. Wash in new Ethanol 100% for 3 minutes.
2. Wash in Ethanol 95% for 3 minutes.
3. Wash in Ethanol 70% for 3 minutes.
4. Wash in 0.05% Tween-20 in PBS for 5 minutes.
5. Wash again in new 0.05% Tween-20 in PBS for 5 minutes.

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4 Microwave:
1.Put the citrate buffer x 1 in a plastic container designated for microwave use.
2.Put the slides in the container.
3.Insert the container into the microwave and activate on maximal energy for 1 minute and on mininal energy for 10 minutes.
4.Stop heating once the buffer boils.
5.Take the contianer out of the microwave, and let it cool down for 15-30 minutes to room temprature. **Do not** transfer the slide directly from the boild buffer to other fluids at room temprature. Wait until proper cool down.

5 Bench, room temprature:
1. Wash in a new 0.05% Tween-20 in PBS for 5 minutes.
2. Wash again in a new 0.05% Tween-20 in PBS for 5 minutes.
3. Take the slide out of the Tween PBS container, and put in the slide staining box.
4. Prepare blocking solution - 3% normal serum of goat/sheep/donkey/other in PBS (make sure it's a different species from the secondary antibody).
5. Gently drop 250-300 ul the blocking solution on the slide.
6. Cover the slide with a paraffin film and incubate for 60 minutes.
7. Remove the paraffin film from the slide. Gently tap the slide on an paper towel in order to dry it.

6 Bench, 4 °C:
1. Add 150-200 ul of a primary antibody diluted in a blocking solution
2. Cover with an new paraffin film.
3. Incubate for 60 minutes at room temprature or 4 °C.
4. At 4°C, the antibody can stay on the slide over-night.

7 Bench, room temprature:
1. Remove the paraffin film.
2. Wash in 0.05% Tween-20 in PBS for 5 minutes.
3. Wash again in a new 0.05% Tween-20 in PBS for 5 minutes.
4. Wash for a thrid time in new 0.05% Tween-20 in PBS for 5 minutes.
5. Add 150-200 ul of Secondary Ab diluted in a blocking solution
6. Cover with paraffin film and incubate 60 minutes in room temprature.
7. Wash in 0.05% Tween-20 in PBS for 5 minutes.
8. Wash again in a new 0.05% Tween-20 in PBS for 5 minutes.
9. Wash for a thrid time in a new 0.05% Tween-20 in PBS for 5 minutes.
10. Wash with DDW for 2 minutes

8 Stain with DAPI (1:2000 in DDW) for 2 minutes.
1.Wash with DDW for 5 minutes.
2.Wash in a new 0.05% Tween-20 in PBS for 5 minutes.
3. Gently tap the slide on an paper towel in order to dry it

9 Cover slides
1.Add a few (2-3) drops of a water-based glue
2. Cover slide with cover-slip
3. Cover slide with an aluminium foil and let them dry over night

10 Proceed to microscopy analysis

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