Isolation of nuclei from paraffin-embedded tissue with subsequent immunostaining

PLOS One

Dec 18, 2019

Dorothe Moellmann[1]

[1]Universitätsklinikum Essen, Institut of Pathology,Germany

1 Works for me dx.doi.org/10.17504/protocols.io.78phrvn

Dorothe Moellmann
Universitätsklinikum Essen, Institut of Pathology,Germany

ABSTRACT

This protocol describes how individual nuclei are isolated from paraffin tissue and then stained immunhistochemically

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0226199

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Schwertheim S, Theurer S, Jastrow H, Herold T, Ting S, Westerwick D, Bertram S, Schaefer CM, Kälsch J, Baba HA, Schmid KW (2019) New insights into intranuclear inclusions in thyroid carcinoma: Association with autophagy and with *BRAFV600E* mutation. PLoS ONE 14(12): e0226199. doi: 10.1371/journal.pone.0226199

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Target Retrieval Solution pH9	S2367	Dako
Dako REAL Antibody Diluent	S2022	Dako
Saponin	A4518.0100	AppliChem
BSA	0163.2	Carl Roth

1 cut 60µm sections from paraffin material and add to an 1,5ml tube

2 dewaxing by addition of xylene; 99%; 96%: 70% Ethanol each for
60:00:00 **Room temperature**
remove and replace after incubation

3 after removing the last rehydration step add Target Retrieval Solution **400 µl**

4 Target Retrieval Solution pH9
by Dako
Catalog #: S2367

centrifugation **5000 rpm , 5min.**

Centrifuge
Benchtop Centrifuge
Eppendorf 5405000441
Any benchtop centrifuge will suffice

discard the supernatant

add Target Retrieval Solution once. **400 µl**

5 add 0,2mm diameter stainless steel beads
place the tube in a homogenizer such as

Homogenizer
Bullet Blender storm 24 NA-BB-15

PDF

homogenize for **02:00:00** max. speed.

There should be no more large pieces of tissue to be seen.

check the result, repeat if necessary

6 The heat induced epitope retrieval (HIER) in the water bath follows.

Place the tube in a preheated water bath **98 °C** **60:00:00**

allow the tube to cool at room temperature for **30:00:00**

7 remove the buffer by centrifugation

5000 rpm , 5min.

discard supernatant

8 add 1x IHC- washing buffer

IHC-Waschpuffer

resuspend pellet by vortexing
repeat step 7

9 prepare the antibody diluent composite

Dako Real Antibody Diluent
0,5% Saponin
2% BSA

Dako REAL Antibody Diluent
by Dako
Catalog #: S2022

Saponin
by AppliChem
Catalog #: A4518.0100
CAS Number: 8047-15-2

BSA
by Carl Roth
Catalog #: 0163.2

10 after centifugation
discard supernatant

start equilibration
add antibody diluent composite **400 µl**
resuspend the pellet
incubate **Room temperature** **30:00:00**

11 Addition of the primary Antibody

The concentration must be determined for each antibody.
Incubation **16:00:00 over Night**
4 °C by agitation

Rotator
Stuart Rotator SB3 SB3

12 centrifugation, **5000 rpm , 5min.**
discard the supernatant

add IHC Waschpuffer

IHC-Waschpuffer

repeat once

13 preparation of the secondary antibody in the antibody diluent composite
in a predetermined concentration

mix well

incubation **Room temperature** **60:00:00**

14 repeat step 7

15 store in Aqua dest **4 °C** if necessary

16 We performed fluorescence immonostaining for 3D reconstruction with laser-scanning microscope
(Leica TCS SP8) afterwards.
If a dapi staining is necessary, it can be connected at the end.

Dapi staining should be performed immediately before microscopy.