

Dec 18, 2019

Isolation of nuclei from paraffin-embedded tissue with subsequent immunostaining 🖘

PLOS One

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

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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 Y	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

७ 60:00:00 ♣ Room temperature

remove and replace after incubation

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



centrifugation <a>\$\oldsymbol{5000}\$ rpm , 5min.



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



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 $\, \odot \, 60:00:00$ allow the tube to cool at room temperature for $\, \odot \, 30:00:00$

- remove the buffer by centrifugation
 - **\$\$5000 rpm**, 5min.

discard supernatant

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

88

Dako REAL Antibody Diluent

by Dako

Catalog #: S2022

88

Saponin

by AppliChem

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

88

BSA

by Carl Roth

Catalog #: 0163.2

10 after centifugation discard supernatant

start equilibration $\mbox{add antibody diluent composite } \begin{picture}(40000) \put(0.000){\mbox{model}} \put(0.000){\mbox{mo$

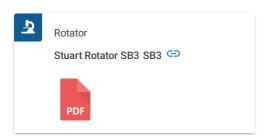
incubate & Room temperature © 30:00:00

11 Addition of the primary Antibody

The concentration must be determined for each antibody.

Incubation © 16:00:00 over Night

§ 4 °C by agitation



centrifugation, **5000 rpm**, **5min**.

discard the supernatant

add IHC Waschpuffer



IHC-Waschpuffer

repeat once

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
- 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.

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