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Isolation of nuclei from paraffin-embedded tissue with subsequent immunostaining

PLOS One

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ABSTRACT

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

OPEN ACCESS

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MATERIALS

STEP MATERIALS

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

Protocol status: Working

Created: Oct 15, 2019

Last Modified: Dec 18, 2019

PROTOCOL integer ID:
28655

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

Target Retrieval Solution pH9 **Dako Catalog #S2367**

4 centrifugation 5000 rpm , 5min.

Equipment

Centrifuge

NAME

Benchtop Centrifuge

TYPE

Eppendorf

BRAND

5405000441

SKU

<https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html>

LINK

Any benchtop centrifuge will suffice

SPECIFICATIONS

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

Equipment	
new equipment	NAME
Homognizer	TYPE
Bullet Blender storm 24	BRAND
NA-BB-15	SKU

homogenize for 02:00:00 max. speed.

Note

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

Dataset	
IHC-Waschpuffer	NAME
http://www.dcs-diagnostics.de/index.php?id=119&item_selectors=item_name%23sale_quantity%23producer&item_specifire=IHC-Waschpuffer%232500%20mL%23DCS	LINK

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 **Dako Catalog #S2022**

Saponin **AppliChem Catalog #A4518.0100**

10 after centrifugation 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

Equipment

new equipment

NAME

Rotator

TYPE

Stuart Rotator SB3

BRAND

SB3

SKU

<http://www.stuart-equipment.de/product.asp?dsl=137>

LINK

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

add IHC Waschpuffer

Dataset

IHC-Waschpuffer

NAME

http://www.dcs-diagnostics.de/index.php?id=119&item_selectors=item_name%23sale_quantity%23producer&item_specifire=IHC-Waschpuffer%232500%20mL%23DCS

LINK

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

Note

Dapi staining should be performed immediately before microscopy.