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Working

Standardized immunohistochemical staining used in the Human Protein Atlas

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Human Protein Atlas

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ABSTRACT

The Human Protein Atlas provides a map showing the distribution and relative abundance of proteins in the human body. All IHC staining in the Human Protein Atlas project are performed using the following standard protocol. The primary antibody dilution is based on titration optimization, the dilution suggested by the Human Protein Atlas can be found under antibody and antigen information for each antibody. When primary antibody originates from other host animals than rabbit, there are some modifications and different secondary antibody is used.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pubmed/22688270

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. Kampf C, Olsson I, Ryberg U, Sjöstedt E, Pontén F. J Vis Exp. 2012 May 31;(63). pii: 3620. doi: 10.3791/3620.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working.

MATERIALS

NAME	CATALOG #	VENDOR
DAB quanto substrate system	Cat# TA-125-QHDX	Thermo Fisher Scientific
UltraVision LP HRP polymer	Cat# TL-125-HL	Thermo Fisher Scientific
Ultra V Block	cat# TA-060-UB	Thermo Scientific
Mayer's hematoxylin plus	cat# 01825	Histolab
Hydrogen peroxid 30%	1.07209.1000	Merck Millipore
NeoClear	1.09843.5000	VWR International
Lithium carbonate	1.05680.0250	Merck Millipore
Pertex	00871.0500	Histolab
Antibody Diluent OP Quanto	TA-125-ADQ	Thermo Fisher Scientific
Tris Buffered Saline & Tween 20 (20x)	TA-999-TT	Thermo Fisher Scientific
Lab Vision™ Tween™ 20 Detergent	cat# TA-125-TW	Thermo Fisher Scientific
Coverslips 24x50mm	631-0146	VWR International
Thermo Scientific™ SuperFrost Plus™ Adhesion slides	J1800AMNZ	Thermo Fisher Scientific

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NAME	CATALOG #	VENDOR
Lab Vision™ PT Module™ Deparaffinization and Heat-Induced Epitope Retrieval Solutions (100X)	TA-250-Pm1x	Thermo Fisher Scientific

MATERIALS TEXT

Wash buffer

9.5L distilled water
500ml Tris Buffered Saline & Tween 20 (20x)
15ml Large Volume Tween 20

Retrieval buffer
5L distilled water
50ml PT Module Buffer 1

SAFETY WARNINGS

Regulations about working with tissue samples may vary between institutions, it is important to be aware about the guidelines before to start any experiment.

3,3′-Diaminobenzidine (DAB) is toxic if swallowed, in contact with skin or if inhaled. It may cause cancer and damage to organs.

BEFORE STARTING

Cut Formalin-Fixed Paraffin-Embedded (FFPE) tissue specimen at 4 µm thickness using a water fall microtome:

HM 355S Automatic Microtome, ThermoFisher Scientific, 905200
Section Transfer System (STS), ThermoFisher Scientific, 771200

Place the section on a superfrost glass slide.

The time the sections can be left in the water depends on the type of paraffin waxed used and the water temperature.
The lab uses paraffin wax from HistoLab Products AB, which have a melting point of 56-58 ° C and we recommend a water bath temperature of 37-39 °C.

Deparaffinization

1 Dry paraffin sections at room temperature overnight.

2 Bake the paraffin sections from 12:00:00 to 24:00:00 at 50 °C .

3 Xylene incubation: incubate slides in xylene for 00:05:00 .
incubate slides in xylene for 00:05:00 .
incubate slides in xylene for 00:01:00 .

4 Ethanol absolute incubation: incubate slides in ethanol absolute for 00:03:00 .

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incubate slides in ethanol absolute for 00:03:00 .

5 96% ethanol incubation & 30%H2O2 (1:100): incubate slides for 00:05:00 .

6 96% ethanol incubation: incubate slides in 96% ethanol incubation for 03:00:00 .

7 80% ethanol incubation: incubate slides in 80% ethanol incubation for 00:03:00 .

8 Distilled water: : incubate slides in distilled water or until antigen retrieval step.

Standard antigen retrieval method

9 Heat the slides immersed in retrieval buffer for 00:04:00 at 125 °C in a pressure boiler (Decloaking chamber model DC2008INTL).

10 After completed boiling, leave the slides in the pressure boiler and let them cool down till 90 °C .
Total time 00:45:00

Immunohistochemical staining program, Autostainer 480

11 Place slides in the Autostainer 480, all the incubations are done at room temperature.

Autostainer 480
ThermoFisher Scientific cat# A80500007

12 Rinse slides in wash buffer.

13 Incubate slides with Ultra V Block for 00:05:00 .

14 Rinse slides in wash buffer go to step #14 once more, total washes 2 .

15 Incubate slides with primary antibody, diluted in antibody Diluent OP, for 00:30:00 .

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16 Rinse slides in wash buffer go to step #16 two times more, total washes 3 .

17 Incubate slides with labeled HRP polymer for 00:30:00 .

18 Rinse slides in wash buffer go to step #18 once more, total washes 2 .

19 Incubate slides with DAB solution for 00:05:00 .

20 Rinse slides in distilled water.

Counterstaining and coversliping

21 Transfer slides in the Autostainer XL.

Autostainer XL

Leica biosystems cat# Leica ST5010 Autostainer XL

22 Counterstain slides with hematoxylin for 00:07:50 .

23 Rinse slides in lithium carbonate water diluted 1:5 from saturated solution for 00:01:00 .

24 Rinse slides in tap water for 00:05:00 .

25 80% ethanol incubation: incubate slides in 80% ethanol for 00:03:00 .

26 96% ethanol incubation: incubate slides in 96% ethanol for 00:03:00 .
incubate slides in 96% ethanol for 00:03:00 .

27 99% ethanol incubation: incubate slides in 99% ethanol for 00:03:00 .
incubate slides in 99% ethanol for 00:03:00 .
incubate slides in 99% ethanol for 00:03:00 .

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28 NeoClear incubation: incubate slides in NeoClear for 00:03:00 .

incubate slides in NeoClear for 00:03:00 .

29 Mount coverslip in each of the slide using Pertex as a mounting media.

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