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Standardized immunohistochemical staining used in the Human Protein Atlas

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Protocol status: Working
We use this protocol in our group and it is working.

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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.

Materials

MATERIALS

- DAB quanto substrate system **Thermo Fisher Scientific Catalog #**Cat# TA-125-QHDX
- UltraVision LP HRP polymer **Thermo Fisher Scientific Catalog #**Cat# TL-125-HL
- Ultra V Block **Thermo Scientific Catalog #**cat# TA-060-UB
- Mayer's hematoxylin plus **Histolab Catalog #**cat# 01825
- Hydrogen peroxid 30% **Merck Millipore (EMD Millipore) Catalog #**1.07209.1000
- NeoClear **VWR International (Avantor) Catalog #** 1.09843.5000
- Lithium carbonate **Merck Millipore (EMD Millipore) Catalog #**1.05680.0250
- Pertex **Histolab Catalog #**00871.0500
- Antibody Diluent OP Quanto **Thermo Fisher Scientific Catalog #**TA-125-ADQ
- Tris Buffered Saline & Tween 20 (20x) **Thermo Fisher Scientific Catalog #**TA-999-TT
- Lab Vision™ Tween™ 20 Detergent **Thermo Fisher Scientific Catalog #**cat# TA-125-TW
- Coverslips 24×50mm **VWR International (Avantor) Catalog #**631-0146
- Thermo Scientific™ SuperFrost Plus™ Adhesion slides **Thermo Fisher Scientific Catalog #**J1800AMNZ
- Lab Vision™ PT Module™ Deparaffinization and Heat-Induced Epitope Retrieval Solutions (100X) **Thermo Fisher Scientific Catalog #**TA-250-Pm1x

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

Troubleshooting

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 start

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.
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.

Equipment

Autostainer 480	NAME
ThermoFisher Scientific	BRAND
cat# A80500007	SKU
https://www.thermofisher.com/order/catalog/product/A80500008	LINK

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 .

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.

Equipment

Autostainer XL	NAME
Leica biosystems	BRAND
cat# Leica ST5010 Autostainer XL	SKU
https://www.leicabiosystems.com/histology-equipment/he-stainers-special-stainers-coverslippers/products/leica-st5010/	LINK

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 .

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.