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Standardized immunohistochemical staining used in the Human Protein Atlas V.1

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Protocol status: Working

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

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Abstract

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**

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.



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 Deparaffinization and hydration are done in xylene and graded ethanol to distilled water.
- 4 During hydration step, block endogeneous peroxidase during ⌚ 00:05:00 in H2O2
[M] .3 % volume
in 95% ethanol.

Standard antigen retrieval method

- 5 Heat the slides immersed in retrieval buffer for ⌚ 00:04:00 at 🌡️ 125 °C in a pressure boiler.
- 6 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

- 7 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}

- 8 Rinse slides in wash buffer.
- 9 Incubate slides with Ultra V Block for  00:05:00 .
- 10 Rinse slides in wash buffer  go to step #10 once more, total washes 2 .
- 11 Incubate slides with primary antibody diluted in antibody diluent for  00:30:00 .
- 12 Rinse slides in wash buffer  go to step #12 two times more, total washes 3 .
- 13 Incubate slides with labeled HRP polymer for  00:30:00 .
- 14 Rinse slides in wash buffer  go to step #14 once more, total washes 2 .
- 15 Incubate slides with DAB solution for  00:05:00 .

16 Rinse slides in distilled water.

Counterstaining and coverslipping

17 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

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

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

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

21 Dehydrate slides in graded ethanol and Neo-Clear.

22 Mount coverslip in each of the slide.

