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Working

Standardized immunohistochemical staining used in the Human Protein Atlas

Version 1

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Human Protein Atlas



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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.

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

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.

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 Percent 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.



Autostainer 480

ThermoFisher Scientific cat# A80500007 [↗](#)

- 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.



Autostainer XL

Leica biosystems cat# Leica ST5010 Autostainer XL [↗](#)

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.



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