Standardized immunohistochemical staining used in the 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.

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SAFETY WARNINGS

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

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

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

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

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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

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**Immunohistochemical staining program, Autostainer 480**

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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**.

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 coverslipping**

21. Transfer slides in the Autostainer XL.

**Autostainer XL**
Leica
*cat# Leica ST5010*

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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 \(00:03:00\).

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

28 NeoClear incubation: incubate slides in NeoClear for \(00:03:00\).

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