

UCP-1 (Abcam ab10983) immunohistochemical protocol

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Abstract

Increasing energy expenditure by stimulating thermogenesis through activation of brown adipose tissue (BAT) and/or induction of browning of white adipose tissue (WAT) is considered a promising strategy to treat/prevent obesity and related metabolic diseases. Whereas WAT is adapted to store energy as triglycerides, BAT produces heat (non-shivering thermogenesis). In brown adipocytes, the uncoupling protein-1 (UCP-1) regulates conversion of energy into heat by uncoupling ATP production from mitochondrial respiration. Also in WAT adaptive UCP-1 positive adipocytes (brown in white: brite or beige) can arise, predominantly in subcutaneous (s) WAT. This browning of WAT is enhanced by exposure to cold temperatures.

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Guidelines

- To dissolve the blocking reagent in TNB, heat TNB blocking buffer to 60°C for 1 hour with stirring. Aliquot over 15 ml tubes. TNB may be stored for up to 1 month at -20°C.
- Pre-incubate the secondary goat anti-rabbit-biotin coupled antibody with the pre-immune serum 1 day before use.
- Harris hematoxylin: 5 sec is a guideline. If solution is fresh, it is best to reduce the time, otherwise sections will be overstained. The Harris hematoxylin work solution has to be replaced every two weeks and filtered daily.

Before start

- Turn on 37°C oven
- Prewarm slide box and 0.1% CaCl₂ solution
- Make sure TNB is stored at -20°C

Protocol

Step 1.

5 min xylene x 2

Step 2.

3 min 100% ethanol x 2

Step 3.

3 min 70% ethanol

Step 4.

3 min 50% ethanol

Step 5.

3 min denatured water (AD)

Step 6.

10 min 1x TBS (37°C)

*10x Tris-buffered-saline (TBS):

- 200 ml Tris-HCl pH 7.5 1 M

- 600 ml NaCl 5 M

- 8 ml Tween

- Stir

- Add AD to 2,000 ml

*1x TBS: mix 100 ml 10x TBS with 900 ml AD

**REAGENTS**

Tween 20 P1379-500ml by [Sigma-aldrich](#)

Step 7.

7 min Trypsin 37°C 2% solution in Trypsin buffer (dilution 1/80)

Trypsin buffer (1L):

-50 ml 1 M Tris-HCl pH 7.8 (0.05 M)

-30 ml 5 M NaCl (0.15 M)

-1g CaCl₂ (0.1%)

-Dissolve in 800 ml AD

-Add AD to 1L

**REAGENTS**

Trypsin from porcine pancreas T7409 by [Sigma-aldrich](#)

Step 8.

5 min TBS

Step 9.

Peroxidase Block: 200 ml methanol + 600 µl H₂O₂ 30% (add just before slides go into methanol solution)

REAGENTS

Hydrogen peroxide 30% 822287.1000 by [Merck Millipore](#)

Step 10.

3 x 5 min TBS

Step 11.

45 min pre-immune goat serum 1/5 in TNB

TNB (1L):

-100 ml 1 M Tris-HCl pH 7.5 (0.1 M)

-30 ml 5 M NaCl (0.15 M)

-5g blocking reagent (0.5%) from TSA BT kit

REAGENTS

✓ Pre-immune goat serum X090710 by Contributed by users

TSA BT amplification kit NEL700001 by [Perkin Elmer](#)

Step 12.

Overnight polyclonal rabbit anti-murine UCP-1 1:200 dilution in TNB at room temperature

REAGENTS

Polyclonal rabbit anti-mouse UCP-1 antibody ab10983 by [Abcam](#)

Step 13.

3 x 5 min TNT

TNT (1L):

-100 ml 1 M Tris-HCl pH 7.5 (0.1 M)

-30 ml 5 M NaCl (0.15 M)

-0.4 ml Tween-20 (0.04%)

Step 14.

45 min goat anti-rabbit-biotin coupled secondary antibody 1/300 + 10% pre-immune mouse serum (all diluted in TNB)

REAGENTS

✓ Polyclonal Goat Anti-Rabbit Immunoglobulins/Biotinylated * 1 ml E043201 by Contributed by

users

Mouse serum M5905-10ML by [Sigma-aldrich](#)

Step 15.

3 x 5 min TNT

Step 16.

30 min streptavidin-coupled peroxidase (PO) 1/100 in TNB (reagent found in TSA BT kit)

Step 17.

3 x 5 min TNT

Step 18.

8 min biotinyl tyramide (BT) 1/50 diluted in amplification diluent (both reagents supplied in TSA BT kit)

Step 19.

3 x 5 min TNT

Step 20.

30 min Streptavidin-PO 1/100 diluted in TNB

Step 21.

3 x 5 min 1x TRIS

10x TRIS (1L):

-500 ml 1M Tris-HCl pH 7.5

-500 ml AD

1x TRIS (1L):

-100 ml 10x TRIS

-900 ml AD

Step 22.

DAB (200 ml 1x TRIS + 65 µl H₂O₂ 30% added fresh)

-Transfer a small amount of DAB to a 0.4 - 0.7 ml microcentrifuge tube (fill only the cone)

-Add DAB to cilinder containing 200 ml TRIS

-Put parafilm on top of cilinder and seal it

-Mix solution by hand until most of the DAB is dissolved

- Filter
- Pour solution into designated container for slides
- Add H₂O₂ to solution just before use.



REAGENTS

3,3-DIAMINO BENZIDINE.4HCl.xH₂O Pure 98% * 5 g 32750-5G by [Sigma-aldrich](#)

Step 23.

3 min AD

Step 24.

Harris Hematoxylin 5 sec

Solution:

- 100 ml Harris Hematoxylin
- 100 ml AD
- 2 ml glacial acetic acid



REAGENTS

Harris Hematoxylin GURR® mercury free 1L 351945S by [Vwr](#)

Step 25.

10 min TAP (running water)

Step 26.

Up and down 50% ethanol (3x)

Step 27.

Up and down 70% ethanol (3x)

Step 28.

Up and down 100% ethanol (3x)

Step 29.

2 x 3 min 100% ethanol

Step 30.

2 x 5 min xylene

Step 31.

Mount coverslip with DPX



REAGENTS

DPX 1.00579.0500 by [Merck Millipore](#)

Warnings

- Xylene: exposure to xylene via inhalation, eyes, ingestion or skin contact. Xylene causes health effects from both acute (<14 days) and chronic (>365 days) exposure. In addition, the health effect will be different depending on the amount a person has been exposed to. Therefore, to avoid exposure to xylene via the skin, special xylene-resistant gloves are worn during xylene handling. Therefore, hydration and rehydration steps and the mounting of the slides are done in a fume hood due to xylene.
- Harris Hematoxylin: special waste container, flammable, eye and skin irritation
- DAB: very carcinogenic, mutagenic compound. Needs to be worked with in a separate fume hood with separate containers, separate waste and separate glassware and utensils.