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Protocol status: Working

UCP-1 (Abcam ab10983) immunohistochemical protocol

PLOS One

Bianca Hemmeryckx¹, Bauters¹,

¹KU Leuven

H. Roger Lijnen¹

Bianca Hemmeryckx

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.

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.

MATERIALS

STEP MATERIALS

Tween 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1379-500ml

Trypsin from porcine pancreas Merck MilliporeSigma (Sigma-Aldrich) Catalog #T7409

Hydrogen peroxide 30% Merck Millipore (EMD Millipore) Catalog #822287.1000

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Keywords: UCP-1, browning,
brown adipose tissue, white
adipose tissue, cold

⊗	Pre-immune goat serum Contributed by users Catalog #X090710
⊗	TSA BT amplification kit Perkin Elmer Catalog #NEL700001
⊗	Polyclonal rabbit anti-mouse UCP-1 antibody Abcam Catalog #ab10983
⊗	Polyclonal Goat Anti-Rabbit Immunoglobulins/Biotinylated * 1 ml Contributed by users Catalog #E043201
⊗	Mouse serum Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5905-10ML
⊗	3,3-DIAMINO BENZIDINE.4HCl.xH2O Pure 98% * 5 g Merck MilliporeSigma (Sigma-Aldrich) Catalog #32750-5G
⊗	Harris Hematoxylin GURR® mercury free 1L VWR International Catalog #351945S
⊗	DPX Merck Millipore (EMD Millipore) Catalog #1.00579.0500
⊗	Tween 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1379-500ml
⊗	Trypsin from porcine pancreas Merck MilliporeSigma (Sigma-Aldrich) Catalog #T7409
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SAFETY 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.

BEFORE START INSTRUCTIONS

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

1 5 min xylene x 2

2 3 min 100% ethanol x 2

3 3 min 70% ethanol

4 3 min 50% ethanol

5 3 min denatured water (AD)

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

Tween 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1379-500ml

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

Trypsin from porcine pancreas Merck MilliporeSigma (Sigma-Aldrich) Catalog #T7409

8 5 min TBS

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

Hydrogen peroxide 30% Merck Millipore (EMD Millipore) Catalog #822287.1000

10 3 x 5 min TBS

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

⊗ Pre-immune goat serum Contributed by users Catalog #X090710

⊗ TSA BT amplification kit Perkin Elmer Catalog
#NEL700001

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

⊗ Polyclonal rabbit anti-mouse UCP-1 antibody Abcam Catalog
#ab10983

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%)

14 45 min goat anti-rabbit-biotin coupled secondary antibody 1/300 + 10% pre-immune mouse serum (all diluted in TNB)

⊗ Polyclonal Goat Anti-Rabbit Immunoglobulins/Biotinylated * 1 ml Contributed by users Catalog
#E043201

⊗ Mouse serum Merck MilliporeSigma (Sigma-Aldrich) Catalog #M5905-
10ML

15 3 x 5 min TNT

16 30 min streptavidin-coupled peroxidase (PO) 1/100 in TNB (reagent found in TSA BT kit)

17 3 x 5 min TNT

18 8 min biotinyl tyramide (BT) 1/50 diluted in amplification diluent (both reagents supplied in TSA BT kit)

19 3 x 5 min TNT

20 30 min Streptavidin-PO 1/100 diluted in TNB

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

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.

3,3-DIAMINOBENZIDINE.4HCl.xH₂O Pure 98% * 5 g Merck MilliporeSigma (Sigma-Aldrich) Catalog #32750-5G

23 3 min AD

24 Harris Hematoxylin 5 sec

Solution:

-100 ml Harris Hematoxylin

-100 ml AD

-2 ml glacial acetic acid

Harris Hematoxylin GURR® mercury free 1L VWR International Catalog
#351945S

25 10 min TAP (running water)

26 Up and down 50% ethanol (3x)

27 Up and down 70% ethanol (3x)

28 Up and down 100% ethanol (3x)

29 2 x 3 min 100% ethanol

30 2 x 5 min xylene

31 Mount coverslip with DPX