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# iDisco immunolabeling in brown adipose tissue (BAT)

In 1 collection

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Optical Clearing of Tissue SPARC

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OPEN ACCESS

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes how to stain Brown Adipose Tissue using iDisco protocol and visualize the result via confocal microscope.

## Attachments

PDF

iDisco.pdf
474KB

## Materials

| | | |
|---|---|---|
| | PBS 10X | Ambion AM9624 |
| | Triton-X100 | Sigma X100-500ML |
| | Tween-20 | Sigma P9416-100ML |
| | DMSO | Fisher D128-4 |
| | Sodium Azide | 58032-100G |
| | Donkey Serum | Jackson Immunoresearch 017-000-121 |
| | Glycine | Sigma G7126-500G |
| | Heparin | Sigma H3393-50KU |
| | Methanol | Fisher A412SK-4 |
| | Hydrogen Peroxide 30% | Sigma 216763-100ML |
| | DiChloroMethane (DCM) | Sigma 270997-12X100ML |
| | (Di)BenzylEhter (DBE) | Sigma 108014-1KG |
| | ParaFormAldehyde 16% | EMS 15710-S |

Reagents and references

| | | |
|---|---|---|
| | Tubes (small samples) | Eppendorf 2ml or USA scientific SealRite 2ml (Cat. 1620-2707) |
| | Tubes (large samples) | Eppendorf 5ml transparent or amber |
| | Tube Revolver/Rotator | Thermofisher 88881001 |
| | Rotating incubator | Scientific Industries Incubator-Genie (SKU: SI-1400) |

Consumables and hardware

## Before start

Starting the process (sample collection) on Tuesday is helpful to avoid doing the longer steps during weekends

## Sample Collection

1 Anesthetize the mouse, perfuse with 20ml Saline and 4% PFA in 0.1M PB

2 Dissect BAT, carefully remove white adipose tissue surrounding the BAT. The two bilateral depots can be left connected or separated, as desired

## Sample Collection

3 Fix in 4% PFA in 0.1M PB at 4°C, overnight with shaking, then RT 1h

4 Wash in PBS with shaking: RT 30min x 3times

## Sample Pretreatment with Methanol

5 Dehydrate with methanol/H2O series: 20%, 40%, 60%, 80%, 100%; at RT for 1h each with shaking

6 Wash further with 100% methanol for 1h at RT and then chill the sample at 4°C with shaking

7 Overnight incubation, with shaking, in 66% DCM / 33% methanol / 1% deionized water at RT

8 Wash twice in 100% methanol at RT with shaking, and then chill the sample at 4°C

9 Bleach in chilled fresh 5% $H_2O_2$ in methanol (1 volume 30% $H_2O_2$ to 5 volumes MeOH), overnight at 4°C with shaking

10 Rehydrate with methanol/H2O series: 80%, 60%, 40%, 20%, PBS; 1h each at RT with shaking

11 Wash in PTx.2 (0.2% TritonX-100 in PBS) for 1h x2 at RT with shaking

## Immunolabeling

12 Incubate samples in Permeabilization Solution (40ml PTx.2 + 10ml DMSO + 1.15g glycine), at 37°C with shaking for 2 days

13 Block in Blocking Solution (42ml PT2.x + 3ml Donkey Serum + 5ml DMSO) at 37°C with shaking for 2 days

14 Incubate with primary antibody in PTwH (0.2% Tween-20 in PBS + 0.1% of 10mg/ml Heparin stock solution in PBS)/5%DMSO/3% Donkey Serum, at 37°C with shaking for 7 days
*Validated primary antibodies
- Beta 3 tubulin (Abcam ab18207. 1:400 dilution)
- Tyrosine Hydroxylase (Millipore AB152, 1:400 dilution)
- Pgp9.5 (Abcam ab108986, 1:400 dilution)

15 Wash in PTwH for 4-5 times (1-2 hours each) until the next day at RT with shaking

16 Incubate with secondary antibody in PTwH/3% Donkey Serum, at 37°C with shaking for 7 days
- Centrifue secondary antibody at 20000g for 10 minutes before use
- Use Amber tube from this step forward
- Wavelength longer than 555 (ex. AF555, AF647) is recommended

17 Wash in PTwH for 4-5 times (1-2 hours each) until the next day at RT with shaking

## Clearing

18 Dehydrate with methanol/H2O series: 20%, 40%, 60%, 80%, 100%; at RT for 1h each with shaking. Ption to leave onernight at this point, is desired

19 3h incubation with shaking, in 66% DCM / 33% methanol / 1% deionized water at RT

20 Incubate in 100% DCM for 15 minutes twice at RT with shaking to wash the methanol

21 Incubate in DiBenzyl Ether (no shaking). The tube should be filled almost completely with DBE to prevent the air from oxidizing the sample. Before imaging, invert the tube a couple of time to finish mixing the solution

## Imaging

22 The cleared sample should be tranferred to a chamber of an appropriate size (we create one with a 3D printer), sealed on a coverglass with Kwik-sil epoxy (VWR). This chamber is then mounted on Attofluor cell chamber (Thermofisher A7816) and filled with DBE

23 We typically use a Nikon Ti Eclipse inverted microscope for scanning confocal microscopy, but a resonance scanner is recommended for faster imaging