iDisco immunolabeling in brown adipose tissue (BAT)

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ABSTRACT

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

EXTERNAL LINK

https://idisco.info/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.cell.com/cell/fulltext/S0092-8674(14)01297-5

PDF

iDisco.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

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 STARTING

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

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

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

14 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