NEURONAL 3D CELL CULTURE MAP2 LABELING KIT

OVERVIEW

Product Name: Anti-MAP2 antibody (ab183830)

Description: Tissue labeling kit for MAP2

Host Species: Rabbit Monoclonal

Size: 1 – 96 well plate or 1 – 384 well plate (replace with 75 µL volumes)

Storage: Store antibody at +4˚C short term (1-2 weeks). Store at -20˚C or -80˚C. Avoid freeze / thaw cycle. Store Visikol HISTO-M at room temperature and Visikol HISTO buffers at +4˚C.

Materials In Kit

- Anti-MAP2 (ab183830)
- Visikol® HISTO-M™ – 30 mL
- Visikol® HISTO™ Penetration Buffer – 30 mL
- Visikol® HISTO™ Washing Buffer 10X – 30 mL
- Visikol® HISTO™ Blocking Buffer – 30 mL
- Visikol® HISTO™ Antibody Buffer – 30 mL

Materials Required

- DAPI
- Methanol (ethanol if FP labeling)
- PBS
- PBS with 1% Triton™ X-100
- DMSO

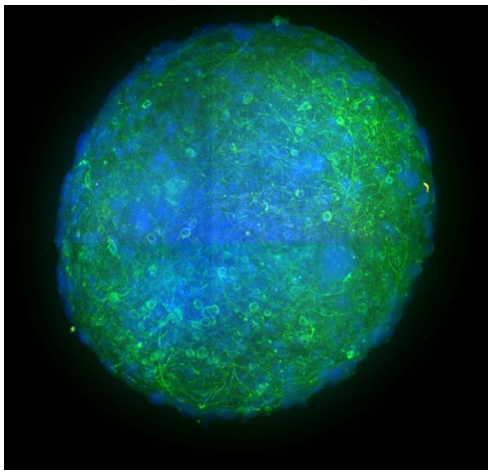

Figure 1. MAP2 in blue, DAPI in green

PROTOCOL

Tissue Processing

1. Fix tissue in your chosen fixative. After fixing for 24 hours, remove and transfer to PBS with 0.05% sodium azide for indefinite storage.
2. Wash tissue in at least 200 µL PBS solution for at least 15 minutes before further procedures to remove traces of fixative.
3. If tissues have been cryopreserved, wash an additional 3-5 times with 200 µL PBS for at least 15 minutes to ensure complete removal of the mounting media.

Permeabilization

1. Wash tissue at room temperature in 200 µL PBS for 15 minutes, 200 µL 50% methanol in DI H_2O, and finally 200 µL 100% methanol for 15 minutes. Samples can be stored in methanol (preferably at 4ºC) indefinitely before proceeding with the next step.
2. Wash samples at room temperature with 200 µL 20% DMSO/methanol for 15 minutes.
3. Wash samples at room temperature with 200 µL 50% methanol for 15 minutes, followed by 200 uL PBS for 15 minutes. CAUTION: During these steps, free-floating 3D cell

cultures will have a tendency to float initially, and must be given enough time to settle to the bottom of the well before the next liquid transfer step.

4. Wash samples at room temperature with 200 µL PBS/1% Triton™ X-100 for 30 minutes.
5. Incubate samples at room temperature in 200 µL Penetration Buffer for 30 minutes with gentle shaking.
6. Block samples in 200 µL Blocking Buffer at 37°C with gentle shaking for 1 hour.
7. Incubate samples with primary antibody dilution prepared in 200 µL Antibody Buffer at 37°C with gentle shaking for 12 hours. To prevent aggregates, we recommend centrifuging or passing the solution through a 0.45 µm syringe filter prior to use. For Anti-MAP2 (ab183830), use a dilution of 1:150.
8. Be sure to dilute the 10x Washing Buffer to a 1x working concentration before using.
9. Wash samples in 200 µL 1x Washing Buffer - 3 times, 15 minutes each time - at 37°C, with gentle shaking.
10. Incubate with secondary antibody prepared in 200 µL Antibody Buffer at 37°C with gentle shaking. Incubate samples for 30 minutes. For Goat Anti-Rabbit Alexa Fluor 488 (ab150077), use a dilution of 1:400. Add desired nuclear label to the secondary antibody buffer at a dilution of 1:100 – 1:5000 depending on the results of your optimization experiments. We recommend using DAPI at 1:1000.
11. Wash in 200 µL 1x Washing Buffer - 3 times, 15 minutes each time, at 37°C, with gentle shaking – followed by a wash with PBS once more. Samples may be kept in this solution indefinitely before proceeding with further steps.

Tissue Clearing

1. Treat tissue at room temperature with 200 µL 50% methanol, followed by 100% methanol for 15 minutes each at room temperature with gentle shaking.
2. Remove methanol from the well.
3. Add 200 µL Visikol HISTO-M to the well and proceed directly to imaging.

Imaging Guidance

Warning! Do not treat specimens with other media after clearing. For optimal results, imaging should be performed directly in Visikol HISTO reagents. Visikol HISTO solutions contain anti-fade agents, and specimens cleared with Visikol HISTO do not need to be mounted in an additional anti-fade media. Other solutions (particularly aqueous media) will cloud the tissue or completely reverse the clearing, interfering with 3D volume imaging. Once the cleared tissues have been successfully imaged, see below for additional information on reversing the clearing to perform downstream assays.

Image 3D tissue cultures directly in the well plate and, if desired, automate the process using a high throughput confocal enabled device.

After 3D imaging, if desired, additional histological or biochemical assays can be performed on Visikol HISTO treated tissue by reversing the clearing. This can be simply done by washing the tissue three times with 200 µL 100% ethanol at room temperature. The tissue can then be processed as any fixed tissue, for SDS-PAGE, Western Blotting, or embedding, sectioning, and staining with H&E, Nissl, IHC or other histological stains.

https://www.youtube.com/watch?v=vtXt1QTA0Z4