Immunofluorescence staining (tissues)

Introduction

Materials

- Antigen Retrieval Buffer
  - 10mM Tri-Soduim Citrate (dihydrate)
  - 5mM EDTA
  - 0,05% Tween 20
  - pH 6,0
- Xylene
- Ethanol
  - 100% / 95% / 70% / 50%
- 1X PBS (Sigma, D-8537)
- Triton-X100
- IF buffer
  - BSA (0.2%)
  - Saponin (0.05%)
  - Sodium azide (0.1%)
  - PH 7.4
  - in PBS
- 10mM ethanolamine. in PBS
- NABH4 (1%) in PBS
- Aurion BSA (Aurion, 900022)
- Donkey Serum (Sigma, S30-100ML)
- Goat Serum (Sigma, S26-100ML)
- Liquid Blocker Pen
- Fluorescene mounting media with DAPI
- SMPD1 / Acid Sphingomyelinase Rabbit anti-Human Polyclonal (aa70-340) Antibody (Lsbio, LS-C334919) (RRID: AB_3086745)
- Monoclonal Anti-Ceramide antibody (Sigma, C8104-50TST) (RRID: AB_259087)

Procedure

1st Day: removing Parafin, rehydrate, ... and incubate 1st antibody

1. For removing the Parafin use Xylene for Rehydration use different concentrations of Ethanol procede the Following Protocol

15 min	Xylene
15 min	Xylene
15 min	Xylene
5 min	Ethanol 100%
5 min	Ethanol 100%
5 min	Ethanol 95%
5 min	Ethanol 70%
5 min	Ethanol 50%
5 min	PBS

2. While Samples are in the PBS prepare Antigen retrieval Buffer and Heat the Presure cooker up to 120°C

   Boil the Samples under Pressure for 35 minutes and afterwards let them cool to room tempereature.

3. Mark your Samples with the Liquid Blocker Pen by drawing circles around them

4. Incubate with 10mM ethanolamine for 5 min at RT

5. Wash 3X 1 min with PBS

6. Incubate with 0.1% Triton-X100 in PBS for 1 min at RT

7. Wash 3X 1 min with PBS

8. Incubate the Samples with 1% NABH4 in PBS by dropping into the circles for 20 minutes (perhaps Liquid Blocker Pen circles need to be drawn again)

9. Wash the samples 3 times with IF-buffer for 5 minutes

10. for ceramide/Smpd1 staining we first block for cermide AB with Aurion 0.5% Aurion BSA, 5% Serum (Depending on source of secondary antibody) in IF-bufferfor 1 hour

11. Dilute ceramide 1st Antibody (usualy 1:20) in Blocking Buffer and incubate overnight at 4°C

2nd Day

12. Wash the Slides times with IF-buffer for 5 minutes

13. block for Smpd1 AB with Aurion 5% Aurion BSA, 5% Serum (Depending on source of secondary antibody) in IF-buffer for 1 hour at RT

14. Dilute Smpd1 1st Antibody (usualy 1:500) in 3% Aurion BSA, 1% Serum (Depending on source of secondary antibody) in IF-bufferforr and incubate for 1 hour at RT

15. Wash the Slides 3 times with IF-buffer for 15 minutes

16. Incubate with the correspondinfg 2nd Antibodies diluted in 1% Aurion BSA, 1% Serum (Depending on source of secondary antibody) in IF-bufferforr for 1 hour at RT

17. Wash the Slides 3 times with IF-buffer for 15 minutes in dark.

18. Mount the coverslip using Fluorescence mounting media with DAPI.