

# Immunohistochemistry staining

---

## Introduction

## Materials

- > Antigen Retrieval Buffer
  - > 10mM Tri-Sodium Citrate (dihydrate)
  - > 5mM EDTA
  - > 0,05% Tween 20
  - > pH 6,0
- > Xylene
- > Ethanol
  - > 100% / 95% / 70% / 50%
- > 1X PBS (Sigma, D-8537)
- > Tween 20 (Sigma, P2287-500ml)
- > Aurion BSA (Aurion, 900022)
- > Liquid Blocker Pen
- > SMPD1 / Acid Sphingomyelinase Rabbit anti-Human Polyclonal (aa70-340) Antibody (Lsbio, LS-C334919) (RRID: AB\_3086745)

## Procedure

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

1. For removing the Parafin use Xylene for Rehydration use different concentrations of Ethanol proceed 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 Pressure cooker up to 120°C

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

3. wash with ddH<sub>2</sub>O for 5 min
4. Mark your Samples with the Liquid Blocker Pen by drawing circles around them
5. block with 3% H<sub>2</sub>O<sub>2</sub> in dark for 20min
6. wash with ddH<sub>2</sub>O for 5 min
7. Wash 2X 1 min with PBS
8. block for Smpd1 AB with Aurion 5% Aurion BSA, in PBST for 1 hour at RT
9. Dilute Smpd1 1st Antibody (usually 1:500) in 3% Aurion BSA in PBST o.n. at 4°C

## 2nd Day

10. Wash the cells with 0.1% Tween 20 in 1X PBS 3 times for 10 minutes each.
11. Incubate with the corresponding 2nd Antibody (DAKO K4003 EnVision+/HRP, Rabbit) for 1 hour at RT
12. Wash the cells with 0.1% Tween 20 in 1X PBS 3 times for 10 minutes each.
13. develop the staining with DAP solution (VEKTOR, SK-4100), Continuously checking the staining development under the microscope and stopping the reaction before there is any background development
14. stop the reaction with ddH<sub>2</sub>O
15. counter stain with Hematoxylin for 60s
16. wash under running tap water for 2min
17. dehydrate with 50%, 70%, 90%, 100% ethanol for 30s each, and then with xylene 3X 5min
18. Mount the coverslip using VectaMount (NC9611543) mounting media.