Immunofluorescence staining of sphingomyelin using lysenin (cells)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- 1X PBS (Sigma, D-8537)
- 4% paraformaldehyde made fresh (Electron Microscopy Sciences, RT15710)
- Aurion BSA (Aurion, 900022)
- Tween 20 (Sigma, P2287-500ml)
- Recombinant Eisenia fetida Lysenin (Biomatik, RPC29534) (stock solution 0.5 mg/ml in PBS)
- Lysenin Antiserum AB (peptanova, 14802-v)
- Donkey anti-rabbit (Cy5) (Jackson I. R, 711-175-152)
- Fluorescence mounting media with DAPI (abcam, ab104139)

Procedure

1. Aspirate medium, wash cells seeded in 8-well chamber slide. When they reach 50-70% coverage, wash quickly with 500 ul of 1X PBS, for 3 times.
2. Fix the cells with 4% paraformaldehyde made fresh in 1X PBS for 15 minutes.
3. Wash with 1X PBS 3 times for 5 minutes each.
4. Block in 1% BSA in PBST (0.1% Tween 20) for 30 min at R.T.
5. incubate with diluted Lysenin (1 µg/ml in blocking buffer) for 90 min at R.T.
6. Wash the cells with 1X PBS 3 times for 5 minutes each.
7. Incubate with Lysenin Antiserum AB (1:300 in blocking buffer) o.n. at 4°C.
8. Wash the cells with 1X PBS 3 times for 5 minutes each.
9. Apply an appropriate fluorophore-conjugated secondary antibody (1:250 in blocking buffer) for 1 h at R.T.
10. Wash the cells with 1XPBS 3 times for 5 minutes each.
11. Mount the coverslip using Fluorescence mounting media with DAPI.

12. image using zeiss microscope.