

Mild vDISCO:

1. Incubate 1 day at 37°C with gentle shaking and in dark in 5mL of permeabilization solution containing 1.5% goat serum (Gibco, 16210072), 0.5% Triton X-100 (AppliChem, A4975,1000), 0.5 mM of methyl- β -cyclodextrin (Sigma-Aldrich, 332615), 0.2% trans-1-acetyl-4-hydroxy-L-proline (Sigma-Aldrich, 441562) and 0.05% sodium azide (Sigma-Aldrich, 71290) in 0.01 M PBS.
2. Incubate 8 days for half brains or 15 days for whole brains at 37°C with gentle shaking and in dark in 4mL of incubation/washing solution (1.5% goat serum, 0.5% Triton X-100, 0.05% sodium azide in 0.01 M PBS). Final volume of the solution is 4mL, add 8-10 μ l of nanoBOOSTER for half brain (1:400-1:500), and double the amount of nanoBOOSTER in case of whole brain.
3. Wash with the same washing solution of point 2 (1.5% goat serum, 0.5% Triton X-100, 0.05% sodium azide in 0.01 M PBS) 3x for 1hour each time at room temperature on a shaker in dark
4. Wash with 0.01M PBS 3x for 1hour each time at room temperature on a shaker in dark
5. Clear with 3DISCO:

50,70,80,100% THF 2h each step, 100% THF overnight, DCM 1-2 hours, BABB