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Brain Clearing (iDISCO)

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PROTOCOL CITATION

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see idisco.info for more details and information regarding antibodies and protocol

MATERIALS TEXT

Solutions:

Note (all of the below solutions contain 0.02% sodium azide—methanol and dcm mixtures do not)

1x PBS

PTx.2 (1L):

50 ml 20x PBS

948 ml ddH₂O

2 ml TritonX-100

PtWH (1L):

50 ml 20x PBS

947 ml ddH₂O

1 ml heparin (10 mg/ml)

2 ml Tween-20

Permeabilization solution (500ml):

400 ml Ptx.2

11.5 g Glycine

100 ml DMSO

Blocking Solution:

42 ml PTx.2

3 ml normal donkey serum

5 ml DMSO

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Day 1:

See materials for solutions to mix:

Dehydrate brains with methanol H₂O series 1h each wash increasing in concentration (20%, 40%, 60%, 80%, 100%) and once again with 100% (6h total)

Incubate brains in 66% dichloromethane (DCM)/33% methanol overnight

Day 2:

Wash the brains twice for 1h each with 100% methanol to rinse out DCM and then chill samples for 10 minutes at 4 C

Chill 5% hydrogen peroxide in methanol and then incubate the samples overnight at 4 C in the solution

Day 3:

Rehydrate the samples using decreasing concentrations of methanol (1h each): 80%, 60%, 40%, 20%, PBS (5h total)

Wash in Ptx.2 twice for 1h (2h total)

Incubate samples in Permeabilization solution overnight at 37 C

Day 4:

Leave samples in Permeabilization solution at 37 C

Day 5:

Incubate samples in Blocking solution at 37 C

Day 6:

Leave samples in Blocking solution at 37 C

Day 7:

Incubate samples in primary antibody (based on what antibody is being used) mixed with 5% DMSO, 3% normal donkey serum in PtWH for 7 days at 37C

Day 14:

Wash samples with PtWH 5x (1h minimum per wash) and leave in the last wash overnight (room temperature)

Day 15: Samples covered with foil from this point forward

Incubate samples in secondary antibody (matched to primary) mixed with 3% normal donkey serum in PtWH for 7 days at 37 C

Day 22:

Wash samples with PtWH 5x (1h minimum per wash) and leave in the last wash overnight (room temperature)

Day 23:

Dehydrate brains with methanol H₂O series 1h each wash increasing in concentration (20%, 40%, 60%, 80%, 100%).

Incubate in a fresh solution of 100% methanol and leave samples overnight

Day 24:

Incubate samples for 3h in 66% DCM/33% Methanol

Incubate samples for 15 minutes 2x in 100% DCM to clear off any methanol residue

Move samples to Dibenzyl Ether without shaking for long term storage

Store at room temperature in the dark until imaged