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Immunofluorescence in PFA-fixed grafted mouse brain slices

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SOX6 mDA differentiation

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol was used to tag protein expression using fluorophore-conjugated antibodies on 20 µm mouse brain slices. Double labeling of proteins and nuclei was succesful on PFA-fixed brain slices.

Materials

PBS1X
PBTx0.3% [Triton-X 0.3% in PBS]
PBTx0.1% [Triton-X 0.1% in PBS]
Blocking Serum Solution (BSS) [Donkey Serum 5%, BSA 1 mg/ml, PBTx0.1%]
Primary antibodies
Secondary antibodies
ReadyProbes Autofluorescence quenching agent
DAPI
Mounting medium

Protocol materials

Fluoromount **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F4680

Troubleshooting

Day one: Primary antibody

19h 35m

1 Place up to eight 20-30 µm slices in wells filled with PBS1X.

For free-floating, 12 well plates were used and slow constant shaking to ensure coating without compromising sample structural integrity.

2 Wash tissue with PBS1X.

PBS1X can be commercial or self-made, but make sure that it is pH 7.4

5m

3 Wash tissue with PBS1X

5m

4 Wash with PBTx0.3%

15m

5 Wash with PBTx0.1%

5m

6 Wash with PBTx0.1%

5m

7 Incubate samples in BSS [Donkey Serum 5%, BSA 1 mg/ml, PBTx0.1%], preferably in plates of smaller wells (e.g 24 well) to optimize volume used.

Blocking solution was prepared for secondary (2ndary) antibodies hosted in donkey. If 2ndary antibody is hosted in goat, add Goat serum 5%. Remember to not use donkey or goat serum if the primary antibody is hosted on any of those serums.

1h

8 Incubate samples in primary antibody solution (in BSS) at 4 °C

Always remember to check antibody host. Use recommended dilution, if no recommendations are there for immunohistochemistry, rule of thumb is 5 x recommendation of immunocytochemistry.

18h

Day two: Secondary antibody

2h 50m

9 Recover slices to 12 well plates. Wash with PBTx0.1%

5m

10 Wash with PBTx0.1% 5m

11 Incubate in secondary solution (in BSS) Room temperature 2h

12 Wash tissue with PBS1X 5m

13 Wash tissue with PBS1X 5m

14 Optional: Incubate samples in ReadyProbes Tissue Autofluorescence quencher (Thermo Scientific, R37630). 10m *

15 Wash with PBS1X 5m *

16 Incubate in DAPI:PBS 1:1000 solution 10m

17 Wash with PBS1X 5m

18 Mount on glass slides with Fluorescent Mounting Media
Fluoromount **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F4680