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DAB staining in PFA-fixed grafted mouse brain slices

Forked from Immunofluorescence in PFA-fixed grafted mouse brain slices

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Roberto Garcia Swinburn[1], Ernest Arenas[1]

[1]Karolinska Institute Stockholm

SOX6 mDA differentiation

Roberto Garcia Swinburn
Karolinska Institute Stockholm

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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 HRP-conjugated antibodies on 20 µm mouse brain slices. DAB 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

3,3′-Diaminobenzidine tetrahydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D5905

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 [Goat serum 5%, BSA 1 mg/ml, PBTx0.1%], preferably in plates of smaller wells (e.g 24 well) to optimize volume used.
If 2ndary antibody is hosted in donkey, use donkey serum
1h

8 Incubate samples in primary antibody solution (in BSS) at 4 °C
We used mouse-anti STEM121 for our protocol.
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 HRP-conjugated secondary solution (in BSS) Room temperature 2h

We used Goat anti-mouse

12 Wash with PBTx0.1% 5m

13 Wash with PBTx0.1% 5m

DAB incubation (in hood) 18m

14 Prepare DAB solution (fresh, protect against light) as manufacturer's instruction

14.1 Dissolve 1 tablet DAB in 15 ml of Tris-buffered saline, pH 7.6

3,3′-Diaminobenzidine tetrahydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D5905

DAB is carcinogenic, so until solution is discarded, all steps should be performed in a protective hood.

14.2 Add 12 µl of fresh 30% hydrogen peroxide

15 Incubate the samples in DAB solution for 8 minutes 8m

16 Wash with PBS1X 5m

17 Wash with PBS1X 5m

Mounting

18 Prepare mounting media (PBS1X: Glycerol 1:1, 0.2% sodium azide). This media lasts up to a year, although it should be discarded if any growths/deposits are observed.

19 Mount stained samples on glass slides using the mounting media.