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Immunofluorescence Staining of Brain Slices

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

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Abstract

Primary antibody labeling to a specific protein using a fluorescence secondary anitbody to label it.

1 Rinse tissues with ice cold 1x PBS
3x @ 10 min

2 Make up **blocking buffer**
1x PBS, containing
3% normal donkey serum
1% bovine serum albumin
1% Gelatin from cold water fish skin (**G7765 Sigma-Aldrich**)
0.1% Triton X-100

3 Block tissues in blocking buffer 1hr on ice

4 Rinse tissues with ice cold **working buffer**
1x PBS, containing
10% Blocking Buffer
0.1~0.5% Triton X-100

5 Make double- or triple- 1stAbs cocktail in Working Buffer

6 Incubate with 1stAbs solution 24 to 72 hrs at 4°C

7 Rinse with ice-cold working buffer
6x @ 5 min

8 Dilute double- or triple- 2nd Abs cocktail in ice-cold working buffer

9 Incubate with 2nd Abs solution 2hr at 4°C

10 Rinse with 1x PBS
3x @ 5 min

11 Incubate tissues with DAPI or Hoechst 33342 solution for 5 minutes

12 Rinse with 1x PBS
3x @ 5 min

13 Mount cover slip using mounting medium