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## Immunofluorescence Staining of Brain Slices

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Tom Deerinck<sup>1</sup>

<sup>1</sup>UCSD

NCMIR@UCSD



National Center for Microscopy and Imaging Research

University of California, San Diego

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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 antibody 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- 1<sup>st</sup>Abs cocktail in Working Buffer
- 6 Incubate with 1<sup>st</sup>Abs solution 24 to 72 hrs at 4°C
- 7 Rinse with ice-cold working buffer  
6x @ 5 min
- 8 Dilute double- or triple- 2<sup>nd</sup> Abs cocktail in ice-cold working buffer
- 9 Incubate with 2<sup>nd</sup> 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