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

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DAB Staining of Fixed Mouse Brain Tissue Sections

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ABSTRACT

This protocol describes steps for immunohistochemical 1 3,3'-Diaminobenzidine (DAB) staining of free floating fixed mouse brain tissue sections.

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MATERIALS

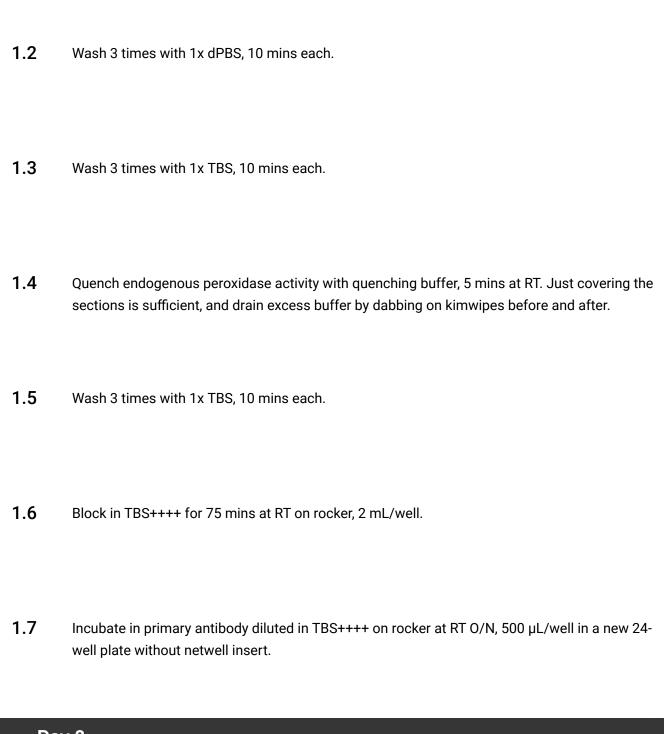
- 24-well netwell insert (Fisher NC9979302)
- dPBS (UCSF Stem Cell Core)
- Tris buffered saline (TBS)
- Methanol
- 30% H₂O₂ (Millipore Sigma H1009)
- Endogenous peroxidase quenching buffer:
 - 10% MeOH, 3% H₂O₂, in 1X TBS
- TBS++++ blocking buffer:
 - 10mL 10X TBS
 - 10mL fetal bovine serum (UCSF Stem Cell Core)
 - 3g BSA (Millipore Sigma A3803)
 - 1g glycine
 - 30mg sodium azide (Millipore Sigma S2002)
 - Bring to 96mL with ddH₂O
 - Store @-20°C in 12mL aliquots
 - Add 500 µL of 10% Triton x-100 (Millipore Sigma 93443) right before use
- Primary antibody (e.g. rabbit anti-TH, Abcam AB152)
- ABC complex VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Rabbit IgG)
 - Manufacturer: Vector Laboratories
 - Cat#: PK-6101
 - Includes biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories

BA-1000-1.5)

- 50X 1 3,3'-diaminobenzidine (DAB) dissolved in 0.1M Tris
 - Millipore Sigma D12384
- 0.1 M Tris, pH 8.0
- Ethanol
- Xylene (ThermoFisher Scientific X51)
- Permount mounting medium (ThermoFisher Scientific SP15)
- Superfrost Plus slides (Fisher 12 550 15)

Day 1

- **1** Blocking and primary antibody incubation.
 - 1.1 Pull all sections into 1x dPBS in 24-well netwells.

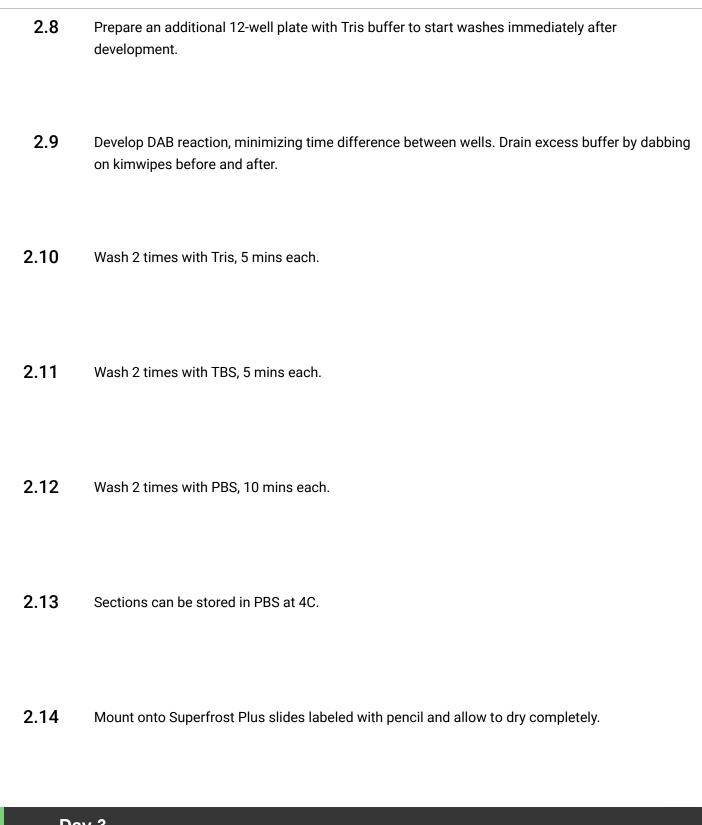


Day 2

2 Secondary antibody incubation and DAB development

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Day 3

3 Dehydration and clearing.