BrdU Staining Graphical Protocol (2023)

BrdU is a thymidine analogue that is commonly used as a marker for cell proliferation. BrdU will incorporate into the DNA of cells undergoing **S-phase** of the cell cycle.

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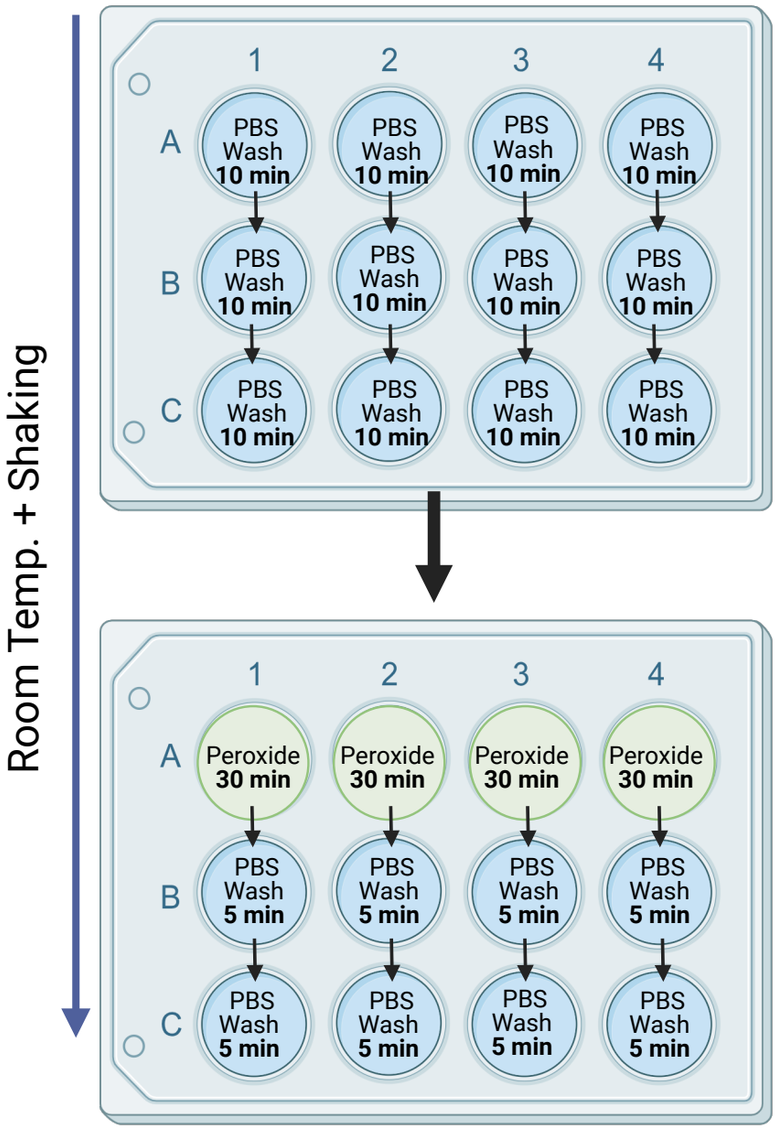
Anti-BrdU-DAB Staining of free floating 50 um brain slices	Date: April 5, 2023

Day 1

Use 12-well plates (one animal per well)

☆ **Turn on Heat Block (37 C)**



Solutions to have pre-made:

1.0 M PBS

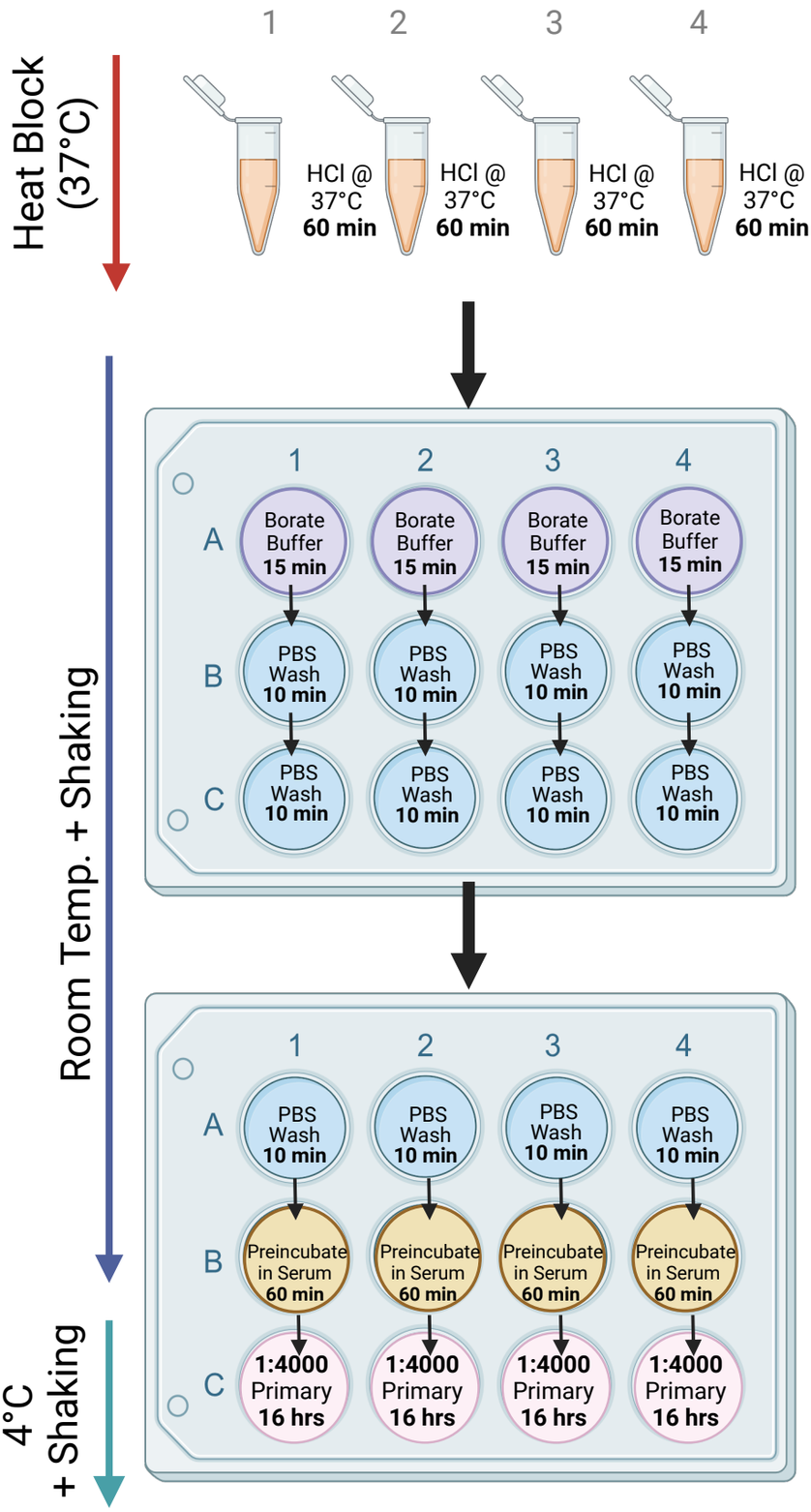
0.25% Triton X-100 & PBS

Peroxide Step (0.6% H_2O_2): Need ~5 mL per well.

Endogenous peroxidases can react with the substrate solution (such as DAB), and cause background staining/false positives. This step blocks endogenous peroxidase activity.

Note: Our lab uses 30% H_2O_2 stock solution.

Day 1 Continued on the Next Page.



Denature DNA in Heat Block with 2N HCl:
Warm Heat Block to 37°C.

(pour acid into water)

Place solution and sample in 1.5 mL eppendorf tubes. Place tubes in the heat block.

⚠ Pressure may build within the tubes as they are heated, so do not close the caps fully when in the heat block.

This step increases the permeability of the cell, and denatures the DNA to make BrdU available for binding.

Neutralize in 0.1 M Borate Buffer (pH to 8.5):
Need ~5 mL per well.

This step neutralizes the acid (HCl) from the previous step.

Preincubate in 3% NGS (normal goat serum) and 0.25% Triton X-100:
Need ~5 mL per well.

This step reduces background staining (serum should be from the same species as the secondary antibody [goat]).

1:4000 Mouse-Anti-BrdU (Millipore Sigma, MAB3424) **in 3% NGS and 0.25% Triton X-100:**
Need ~1 mL per well.

4°C + Shaking for 16 hrs.

End of Day 1.

Day 2

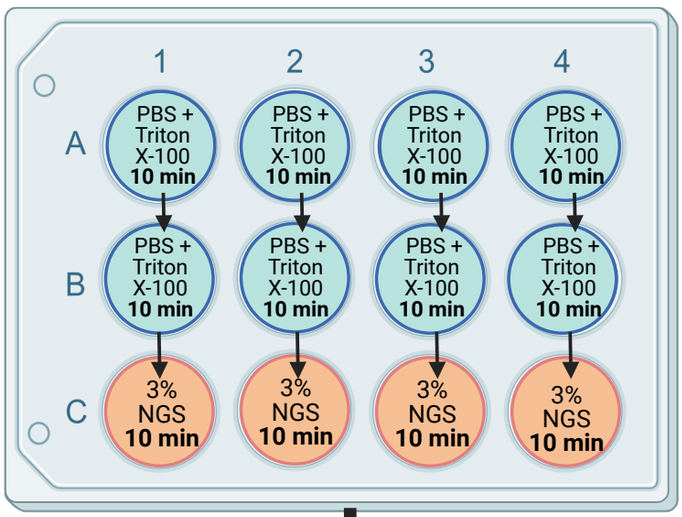
PBS with 0.25% Triton X-100 Rinse: Need ~5 mL per well.

Triton X-100 is a detergent, and is used to increase cell permeability through the solubilization of the lipid membrane.

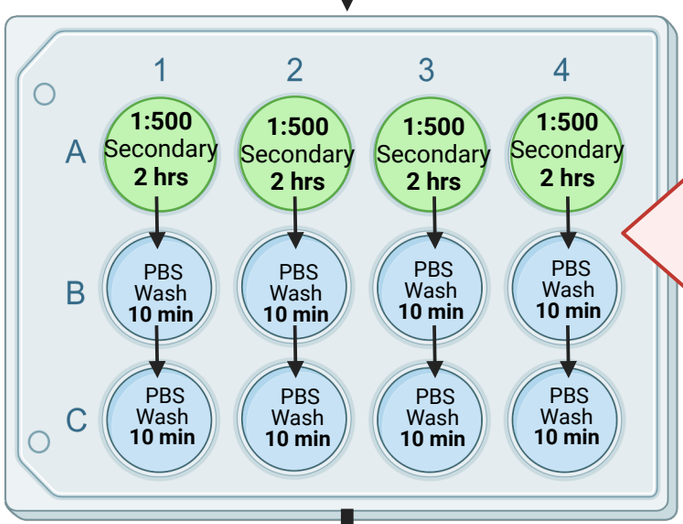
3% NGS in PBS Rinse: Need ~5 mL per well.

This step reduces background staining by preventing non-specific binding of secondary antibodies.

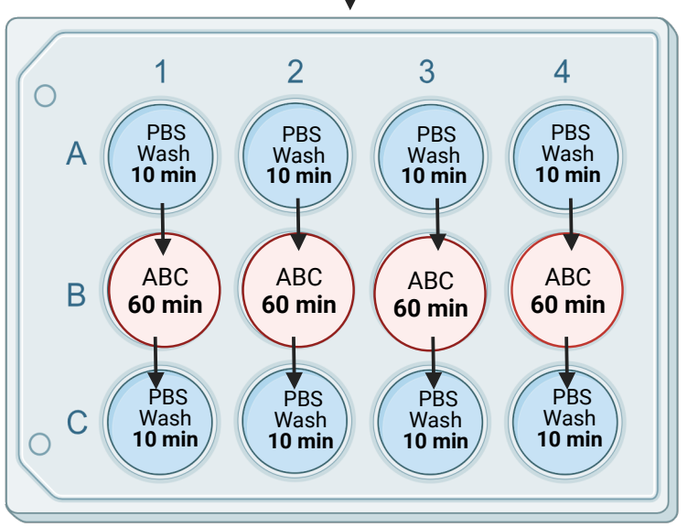
Room Temp. + Shaking



1:500 Goat-Anti-Mouse (Millipore Sigma, B6649) **in 3% NGS and 0.25% Triton X-100: Need ~1 mL per well.**



Make ABC Solution here and incubate in the fridge for **30 min!**

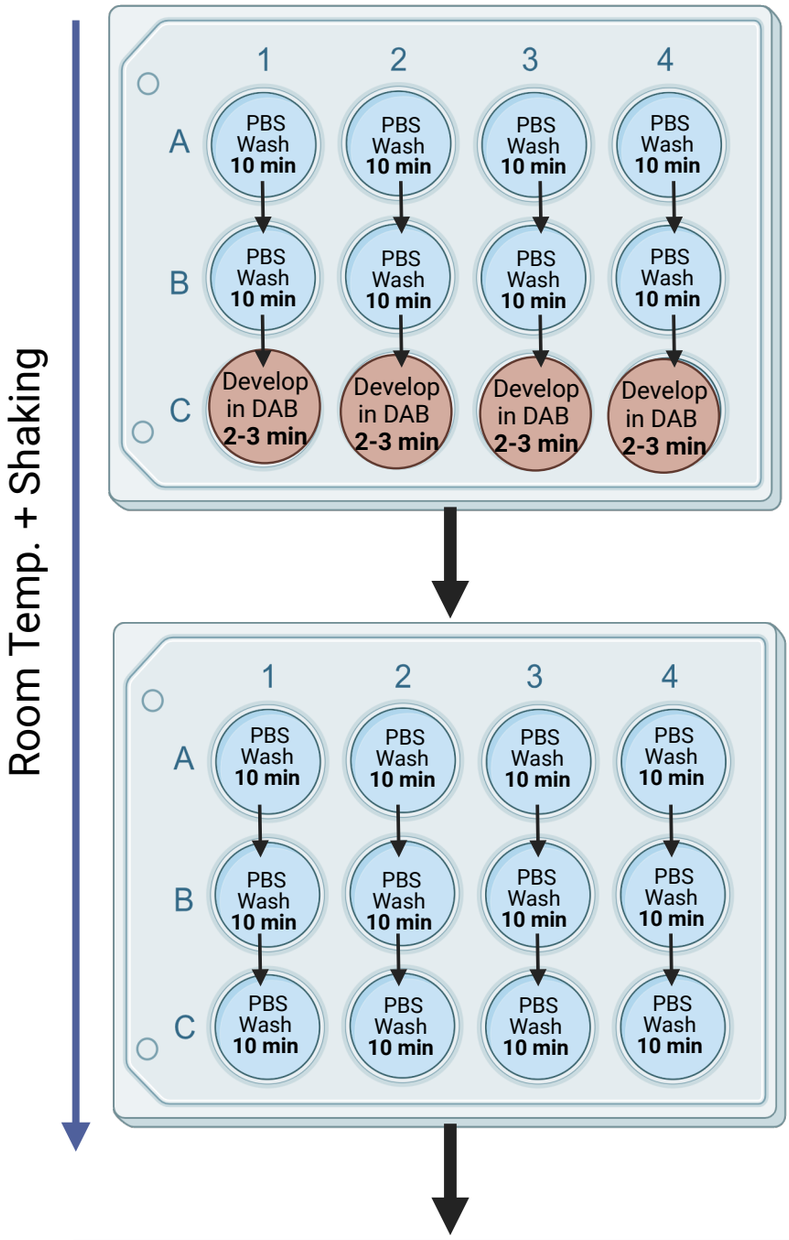


Making the ABC:
Need ~ 2mL per well.
We use the Vectastain Elite ABC kit.

1. Fill falcon tube w/ PBS to desired amount.
2. Add 2 drops of reagent A and 2 drops of reagent B for every 5 mL.
3. Vortex solution.
4. Place the falcon tube in the fridge and allow to incubate for exactly 30 minutes.

Adds enzyme complexes to secondary antibodies. When chromogenic substrate is added (DAB step), these enzymes will react with the substrate and release a coloured precipitate.

Day 2 Continued on the Next Page.



Develop in DAB:
~5 mL per well.

1. Fill falcon tube w/ dH_2O to desired amount.
2. Add 2 drops of reagent A, 2 drops of reagent C, and 4 drops of reagent B for every 5 mL of dH_2O.
3. Vortex for 15 sec and add to wells.

⚠ This procedure must be performed in the fume hood, and anything that comes into contact with DAB needs to be deactivated or disposed of appropriately.

DAB is a chromogenic substrate that will release a brown precipitate with enzymatic activity.

Mounting The Brains:

1. Wet mount in 1x PBS and leave to dry (*place a cover overtop to prevent dirt from falling on the slides*).
2. Dehydrate tissue (*be sure to use fresh solutions!*):
   i) 50% EtOH (5 min)
   ii) 70% EtOH (5 min)
   iii) 100% EtOH (5 min)
   iv) Citrisolv (5 min)
3. Coverslip with permount in fumehood, and leave to dry overnight.

References:

1. Neale, K.J., Reid, H.M.O., Sousa, B., McDonagh, E., Morrison, J., Shultz, S., Eyolfson, E., Christie, B.R. Repeated Mild Traumatic Brain Injury Causes Sex-specific Increases in Cell Proliferation and Inflammation in Juvenile Rats, 26 June 2023, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-3064324/v1]
2. Kannangara, T.S., Webber, A., Gil-Mohapel, J. and Christie, B.R. (2009), Stress differentially regulates the effects of voluntary exercise on cell proliferation in the dentate gyrus of mice. Hippocampus, 19: 889-897. https://doi.org/10.1002/hipo.20514
3. Yau S-Y, Li A, Zhang E-D, et al. Sustained Running in Rats Administered Corticosterone Prevents the Development of Depressive Behaviors and Enhances Hippocampal Neurogenesis and Synaptic Plasticity without Increasing Neurotrophic Factor Levels. *Cell Transplantation*. 2014;23(4-5):481-492. doi:10.3727/096368914X678490
4. Ernst, C., Christie, B.R. Isolectin-IB 4 as a vascular stain for the study of adult neurogenesis.

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