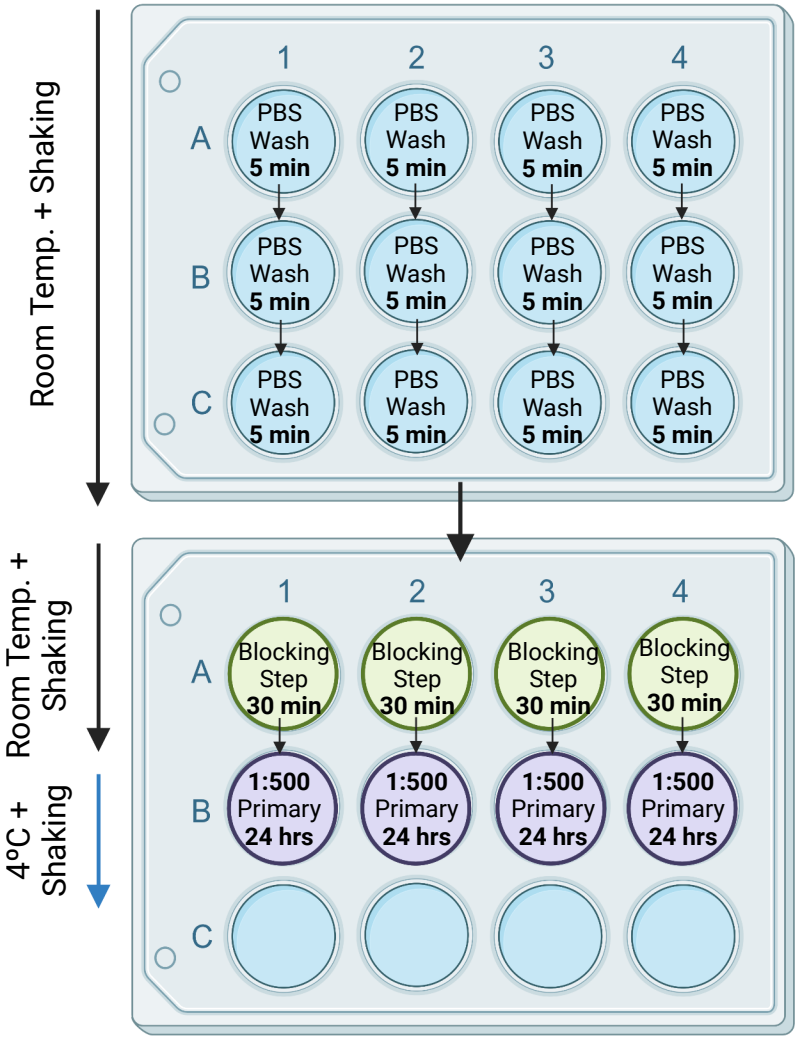
c-Fos Troubleshooting 2023

Jamie Morrison, Adapted from Hannah Reid & Jason Snyder's Protocol

c-Fos: An immediate early gene that can be used to indicate activated neurons.
Purpose: To determine the best protocol for examining c-Fos expression in aged rat hippocampi.

Day 1



Details

Wells: Use one well per animal

Pre-make: 0.5% Triton X-100 in PBS
-> 250 mL } 1.25 mL Triton X-100 + 248.75 mL PBS

PBS Washes

Details: Use 1x PBS

Purpose: Phosphate-buffered saline (PBS) is of neutral pH and is isotonic to biological tissue. PBS is used to wash off any unbound or nonspecifically bound substances from tissue.

Blocking Step

Details: Use 3% Normal Goat Serum (NGS) and 0.5% Triton X-100 (Tx) in PBS.
-> 8 mL } 240 µL NGS + 7.76 mL PBS-Tx

Purpose: Tx is used to permeabilize cell membranes and allow antibodies to access antigens. Goat serum is used to block non-specific antibody binding and minimize background staining by binding to non-specific substrates.

Primary Antibody

Details: Using c-Fos (9F6) Rabbit mAb #2250; 1:500 dilution in blocking solution (3% NGS and 0.5% Triton X-100 in PBS)
-> 4 mL } 8 µL Primary + 120 µL NGS + 3.872 mL PBS-Tx

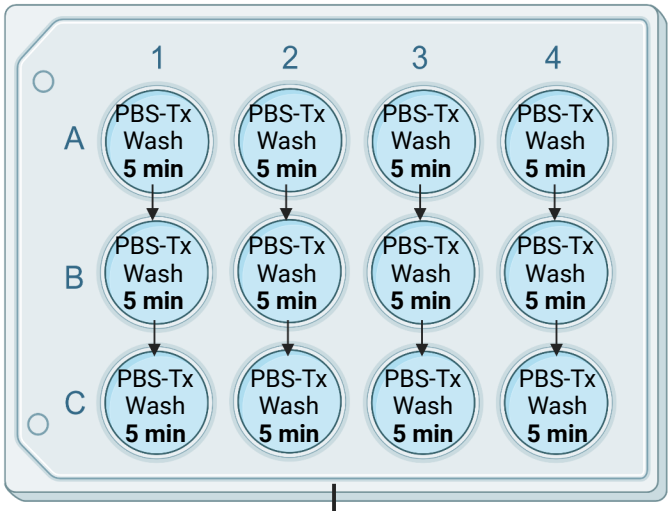
Purpose: The primary antibody is used to specifically bind to c-Fos antigens.

Incubate at 4°C, shaking for 24 hours

End of Day 1

Day 2

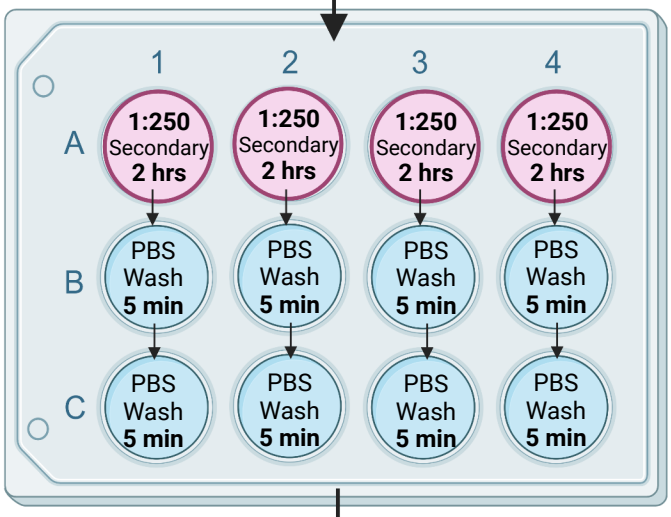
Room Temp. + Shaking



PBS-Tx Washes

Purpose: Wash off remnants of 1° antibody solution. Tx used to permeabilize cell membranes and allow antibodies to access antigens.

Room Temp. + Shaking

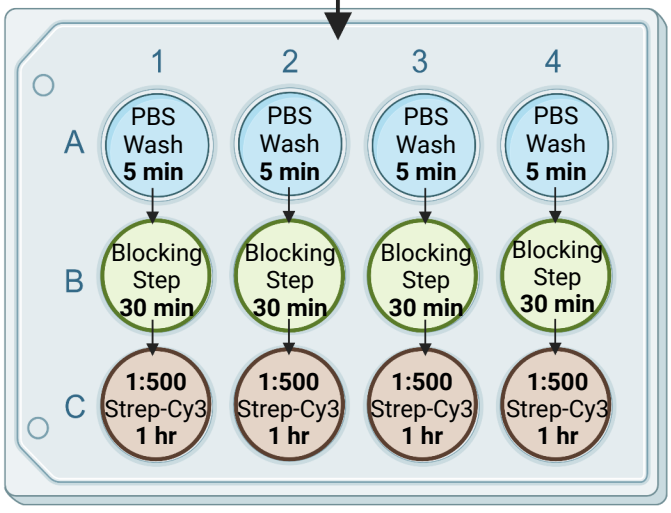


Secondary Antibody

Details: Using Goat Anti-Rabbit IgG H&L (Biotin) (ab6720); 1:250 dilution in blocking solution (3% NGS and 0.5% Triton X-100 in PBS)
-> 4 mL } 16 mL Secondary + 120 µL NGS + 3.864 mL PBS-Tx

Purpose: Add a biotinylated secondary which allows you to conjugate the streptavidin-Cy3 complex and amplify the signal from the 1° and 2° antibody.

Room Temp. + Shaking in Dark



Blocking Step

Details: Use 3% Normal Goat Serum (NGS) and 0.5% Triton X-100 (Tx) in PBS.
-> 8 mL } 240 µL NGS + 7.76 mL PBS-Tx

Purpose: Tx is used to permeabilize cell membranes and allow antibodies to access antigens. Goat serum is used to block non-specific antibody binding and minimize background staining by binding to non-specific substrates.

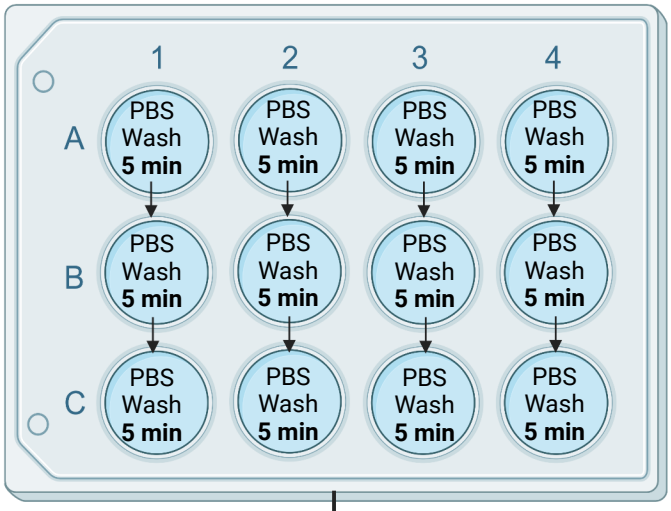
Streptavidin-Cy3

Details: Use Streptavidin-Cy3; 1:500 dilution in blocking solution (3% NGS and 0.5% Triton X-100 in PBS)
-> 4 mL } 8 µL Streptavidin-Cy3 + 120 µL NGS + 3.872 mL PBS-Tx
-> Streptavidin-Cy3 is light sensitive, ensure incubation is in the dark (e.g. wrap well plate in foil)

Purpose: Streptavidin binds to the biotinylated secondary and allows for fluorescence due to the conjugated fluorescent dye, Cy3.

Day 2 Cont'

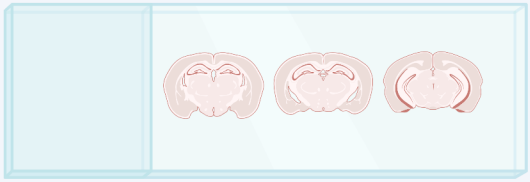
Room Temp. + Shaking



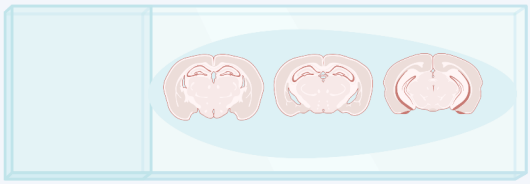
PBS-Tx Washes

Purpose: Wash off remnants of 1° antibody solution. Tx used to permeabilize cell membranes and allow antibodies to access antigens.

Mount Tissue on Slides



1:1000 Hoechst 15 min

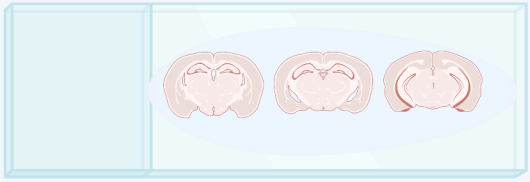


Hoechst

Details: Use Hoechst 33342; 1:1000 dilution in 1x PBS -> 5 mL } 5 µL Hoechst + 4.995 mL PBS

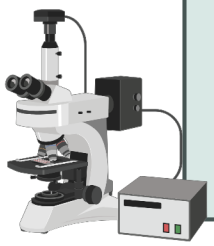
Purpose: Hoechst is a fluorescent dye that stains nuclei by binding to the minor grooves of DNA. Hoechst is used as a counterstain.

3 x 5 min PBS Washes

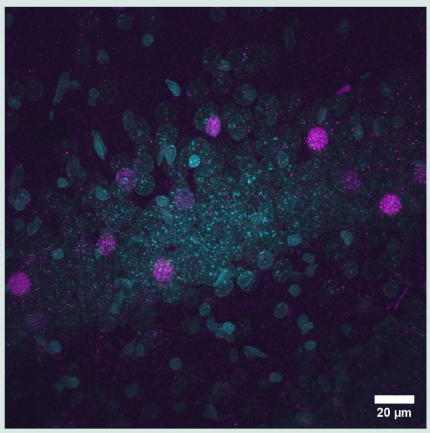


Final Steps

1. Let slides partially dry for 10-15 min
2. Coverslip with Fluoromount-G mounting medium
3. Visualize with a fluorescent microscope



60x stack image of c-Fos positive cells (magenta) in the rat granule cell layer.



Done!