

# IHC Protocol – cFos (red) & EYFP (green)

## Day 0

- 1) Slice brains to 50 micron sections, store in 1x PBS (~6 sections/1 mL well)

## Day 1

- 1) Prepare PBST such that the solution = 1x PBS + 0.2% Triton
  - a. **1x PBS** (1L): **900mL** dH<sub>2</sub>O + **100mL** 10x PBS
  - b. **1x PBS + 0.2% Triton** (1L): **998mL** 1x PBS + **2mL** Triton
- 2) Add normal goat serum (NGS) to PBST such that the final solution contains 5% NGS.
  - a. **PBST-NGS** (20mL): **19mL** 1x PBS + **0.2% Triton** + **1mL** 100% NGS
- 3) Fill each well (~1mL/well) with **PBST+NGS** & add slices
- 4) Place plate on shaker at room temp. for 1 hour (blocking stage to prevent non-specific binding)
- 5) After an hour, transfer slices to new set of wells containing the primary antibody solution. Make the following dilutions in **PBST-NGS**:
  - a. 1:5000 **rabbit anti-cFos** (SySy, 20 mL): **4uL** rabbit anti-cFos
  - b. 1:1000 **chicken anti-GFP** (ThermoFisher, 20mL): **20uL** chicken anti-GFP
  - c. *If making 20mL*: **4uL** **rabbit anti-cFos** + **20uL** **chicken anti-GFP** + **19mL**, **976uL** **PBST-NGS**
- 6) Once transferred, place in cold room on shaker for 24 hr (overnight)

## Day 2

- 1) Wash 3 times with **PBST** for 10 min; place plate on shaker for 10 min
- 2) Transfer slices to new set of wells containing the secondary antibody solution. Make the following dilutions in **PBST + 5% NGS** (*see Day 1, 2A*):
  - a. **1:200 Alexa 555 anti-rabbit** (ThermoFisher)
  - b. **1:200 Alexa 488 anti-chicken** (ThermoFisher)
  - c. *If making 20mL*: **100uL** **Alexa 555 anti-rabbit** + **100uL** **Alexa 488 anti-chicken** + (**19mL**, **800uL** **PBST-NGS**)
- 3) Place plate (covered in aluminum foil) on shaker at room temp. for 1 hour
- 4) Repeat PBST washes 3x
- 5) Mount slices on slides using VectaShield + DAPI as mounting medium

## Antibodies

- 1) GFP Tag Polyclonal Antibody (chicken) - ThermoFisher - catalog # A10262
- 2) anti c-Fos (rabbit) polyclonal affinity purified 50µg - Synaptic Systems (SySy) - catalog # 226 003
- 3) Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 - ThermoFisher - catalog # A11039
- 4) Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 - ThermoFisher - catalog # A21429