

c-Fos Immunofluorescence Troubleshooting 2023  
Written Protocol

**What it is:**

c-Fos is an immediate early gene that is expressed upon changes in neuronal activity. Therefore, studies have used immunohistochemistry detection of c-Fos to examine patterns of neuronal activity.

**Immunohistochemistry Protocol Day 1:**

- 3x5 min PBS washes
- 30 min blocking in 0.5% Triton X-100 in PBS & 3% Normal Goat Serum (NGS)
- 24 hour 1:500 c-Fos primary incubation at 4°C

**Immunohistochemistry Protocol Day 2:**

- 3x5 min 0.5% Triton X-100 in PBS washes
- 2 hour 1:250 biotin goat anti-rabbit 2<sup>o</sup> incubation at room temperature
- 3x5 min PBS washes
- 30 min blocking in 0.5% PBS-Tx & 3% NGS
- 1 hour 1:500 streptavidin-cy3 incubation at room temperature in dark
- 3x5 min PBS washes
- Mount slices
- 15 min 1:1000 hoechst counterstain
- 3x5 min PBS washes
- Coverslip with fluoromount-G