**Fluorescent cFos Immunohistochemistry Protocol (free-floating tissue; updated January, 2016)**

**Experimental info Reagent info (manufacturer, source)**

Researcher(s): 1° antibody: 1:2000 Rabbit-anti-phospho-cFos (CST, 5348)

Experiment: 2° antibody: 1:500 Goat-anti-rabbit AF488 (Abcam, ab150077)

Animal #(s): PBS (0.01M); 10% Triton X; serum

**Procedure: Day 1 (date )**

1. Washes
   1. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
2. Non-specific tissue blocking
   1. 2000 l /well, 1 hours, room temperature, gentle agitation; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.02 serum
      * **VTriton X: l** = Vtotal  l x 0.001 10% Triton X
      * **VPBS: l** = Vtotal l– Vserum  l – VTriton X  l
3. 1° antibody incubation
   1. 2000 l /well, 48 hours, 4°C, gentle agitation, *dark*; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.02 serum
      * **VTriton X: l** = Vtotal  l x 0.005 10% Triton X
      * **V1° Ab : l** = Vtotal  l x 0.0005 1° antibody
      * **VPBS: l** = Vtotal l– Vserum  l – VTriton X l – V1° Ab l

**Procedure: Day 2 (date )**

1. Washes
   1. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
2. 2° antibody incubation
   1. 2000 l /well, 1 hour, room temperature, gentle agitation; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.015 serum
      * **V2° Ab : l** = Vtotal  l x 0.002 2° antibody
      * **VPBS: l** = Vtotal l– Vserum  l – V2° Ab  l
3. Washes
   1. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 5 minutes, room temperature, gentle agitation; **start time**:
4. Mounting and image
   1. Mount tissue and coverslip with DAPI mounting medium
   2. Image under brightfield