**mCitrine Immunohistochemistry Protocol (free-floating tissue; updated August, 2015)**

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

Researcher(s): 1° antibody: 1:1000 chicken anti-GFP (ab13970 Krashes Ab Box 3)

Experiment: 2° antibody: 1:500 donkey anti-chicken AF488 (Krashes Fluorescent 2° Ab

Animal #(s): PBS (0.01M); Triton X-100; goat serum

**Procedure: Day 1 (date )**

1. Washes
   1. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
2. Non-specific tissue blocking
   1. 2000 l/well, 2 hours, room temperature, gentle agitation; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.04 goat serum
      * **VTriton X: l** = Vtotal  l x 0.004 Triton X-100
      * **VPBS: l** = Vtotal l– VBSA  l – VTriton X  l
3. 1° antibody incubation
   1. 2000 l/well, 24 hours, 4°C, gentle agitation, *dark*; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.04 goat serum
      * **VTriton X: l** = Vtotal  l x 0.004 Triton X-100
      * **V1° Ab : l** = Vtotal  l x 0.002 1° antibody
      * **VPBS: l** = Vtotal l– VBSA  l – VTriton X  l – V1° Ab  l

**Procedure: Day 2 (date )**

1. Washes
   1. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
2. 2° antibody incubation
   1. 2000 l/well, 2 hours, room temperature, gentle agitation; **start time**: ;
      * **Vserum: l** = Vtotal  l x 0.04 goat serum
      * **VTriton X: l** = Vtotal  l x 0.004 Triton X-100
      * **V2° Ab : l** = Vtotal  l x 0.002 2° antibody
      * **VPBS: l** = Vtotal l– Vserum – VTriton X  – V2° Ab
3. Washes
   1. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   2. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   3. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
4. Mounting and image
   1. Mount tissue and coverslip with DAPI mounting medium
   2. Image under DAPI (461) and FITC (488) fluorescence