**HA Tag Immunohistochemistry Protocol (free-floating tissue; updated June, 2015)**

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

Researcher(s): 1° antibody: 1:100 mouse anti-HA mAb (BioLegend, 901514)

Experiment: 2° antibody: 1:500 AF647 (TRITC) goat anti-mouse (Life Tech, A-21235)

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

**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**: ;
      * **VBSA: l** = Vtotal  l x 0.04 BSA
      * **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. 200 l /tube, 24 hours, 4°C, gentle agitation, *dark*; **start time**: ;
      * **VBSA: l** = Vtotal  l x 0.04 BSA
      * **VTriton X: l** = Vtotal  l x 0.004 Triton X-100
      * **V1° Ab : l** = Vtotal  l x 0.01 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. 200 l /tube, 2 hours, room temperature, gentle agitation; **start time**: ;
      * **VBSA: l** = Vtotal  l x 0.04 BSA
      * **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– VBSA  l – VTriton X  l – V2° Ab  l
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 TRITC (647) fluorescence