**FLAG Tag Immunohistochemistry Protocol (frozen tissue; updated November, 2015)**

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

Researcher(s): 1° antibody: 1:500 mouse anti-FLAG pAb (Sigma, F1804)

Experiment: 2° antibody:

Animal #(s): 1X PBS (0.01M); Triton X-100; BSA; non-fat dry milk

**Procedure: Day 1 (date )**

1. Tissue fixation
   1. dry slides using a slide warmer
   2. fix tissue in NBF, 10 minutes, room temperature, gentle agitation; **start time:**
2. 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**:
3. Non-specific tissue blocking
   1. 200 l/slide, 30 minutes, room temperature, gentle agitation; **start time**: ;

* **Blocking solution = 1% BSA, 0.2% milk, 0.3% Triton-X in PBS**

1. 1° antibody incubation
   1. 200 l/slide, 24 hours, 4°C, gentle agitation, *dark*; **start time**: ;

* **1° antibody = 1:500 in blocking solution**

**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/slide, 2 hours, room temperature, gentle agitation; **start time**: ;

* **2° antibody = 1:500 in blocking solution**

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. Mounting and image
   1. Mount tissue and coverslip with DAPI mounting medium
   2. Image under fluorescence