**Anti-FLAG Immunohistochemistry Protocol (free-floating tissue; updated December, 2015)**

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

Researcher(s): 1° antibody: 1:200 mouse anti-FLAG pAb (Sigma, F1804)

Experiment: 2° antibody: 1:500 Donkey-anti-mouse (Alexa555; Invitrogen)

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

**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. 2.0 ml/well, 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. 2.0 ml/well, 24 hours, room temperature, gentle agitation, *dark*; **start time**: ;

* **1° antibody = anti-FLAG Ab with 0.3% Triton-X in PBS**
* **1° control = 0.3% Triton-X in PBS**

**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. 2.0 ml/well, 2 hours, room temperature, gentle agitation; **start time**: ;

* **2° antibody = donkey-anti-mouse Ab with 0.3% Triton-X in PBS**
* **2° control = 0.3% Triton-X in PBS**

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 (DAPI = 405/DAPI, GFP = 488/FITC, anti-FLAG = 555/TRITC)