### Day 1

1. **60 µm sections**

2. **wash in PBS, 4 x 10min**

3. heat mediated antigen retrieval in citrate buffer
   - citrate buffer: 10mM sodium citrate buffer, pH 6.0, adjust with citric acid
   - 3a. 5min in 10mM citrate buffer, pH 6.0
   - 3b. 3h in 10mM citrate buffer, pH 6.0 at 80°C (water bath)
   - 3c. let sections cool down in citrate buffer, 30min at RT

4. **blocking step: 2h, room temperature, on a shaker**
   - blocking solution: PBS, 0.5% Triton X-100, 10% goat serum

5. **rinse briefly in PBS, 1x**

### Day 2

1. **rinse in PBS, 4x 10min**

2. secondary antibody anti-mouse 568 or 488 (highly cross absorbed), 1:750 in carrier solution
   - carrier solution: PBS, 0.5% Triton X-100, 1% goat serum
   - incubate for 6h, room temperature, on a shaker, in the dark

3. **rinse in PBS, 4x 10min**

4. mount sections on object slides, air dry sections, coverslip using Vectashield mounting medium
   - store mounted sections at 4°C