**Multiplex Solutions**

Bleach Base

1. 1g Sodium Carbonate
2. 150mL Pure Water
3. 17uL NaOH 10M

Bleach Solution

This solution is 3% H2O2 diluted in the Bleach Base.

1. 5mL Bleach Base
2. 625uL 30% H2O2

Antibody Dilution Stock

All antibody dilutions are done in this stock.

1. Odyssey blocking buffer
2. 0.01% triton x-100

**Protocol**

1. Stain tissue with Hoescht at 1:10k dilution for 5 minutes
2. Wash 2x with PBS, 5 min between.
3. First cycle = primary, secondary cycle
   1. Incubate primary for 45 minutes at RT
   2. Wash 2x with PBS with 5 minutes between washes
   3. Incubate secondary for 45 minutes at RT
   4. Wash 2x with PBS with 5 minutes between washes
4. Image with microscope
5. Remove PBS and add bleach solution for 30 minutes onto tissue.
6. Wash 2x with PBS with 5 minutes between washes
7. Incubate with next cycle’s antibodies (directly conjugated) for 45 minutes
8. Wash 2x with PBS with 5 minutes between washes
9. Image with microscope
10. Repeat steps 5-9 until all cycles are completed.

**Look out for**

* Imaging assumes it is in focus. Please update focus manually beforehand.
* Objective must be risen up when doing the bleach cycle.