

## Whole Mount Antibody Staining

(optimized for E14.5 mouse limbs)

(modified by Kelsey Lewis and Gabrielle Kardon from Mike Klymkowski's protocol)

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### **Specimen Preparation**

1. Fix overnight in 4% PFA (paraformaldehyde made up in 1x PBS), rocking at 4°C.
2. Rinse 3 times with PBS
3. Bleach overnight with Dent's bleach, rocking at 4 degrees C.
  - If the embryo needs to be skinned, do this before bleaching.
4. Rinse 5 times with MeOH
5. Fix in Dent's fix at 4°C
  - For thicker (older) mouse limbs, I leave them in Dent's fix for weeks or months) to improve penetration.
  - For younger embryos, a week or a couple weeks should be sufficient.
  - The minimum is 24 hours (but I have never done this).
  - I leave the embryo whole until I'm ready to start 1°, and then remove individual limbs for staining.

### **Antibody Staining**

#### **Primary Antibody**

1. Rinse 3 times in PBS
2. 3 x 1 hour PBS washes
3. Block 1 hour in blocking solution
4. Primary antibody(ies) in blocking solution for 3 nights at room temperature gently rocking/rotating

#### **Secondary Antibody**

1. Rinse 3x in PBS
2. 5 x 1 hour PBS washes
3. Secondary antibody(ies) in blocking solution for 3 nights at room temperature gently rocking/rotating, keep in dark by covering

### **Clearing and imaging**

1. Rinse 3 x 5 min in PBS
2. 3 x 1 hour PBS washes
3. Postfix: 1 hour 4% PFA \*\*\*
4. Rinse 3 times in PBS
5. 2 or 3 one hour PBS washes
6. Replace half of PBS with MeOH, let stand 5 min
7. 4 MeOH long rinses, 10 minutes each (or 3 x 20 minutes MeOH)
8. 1/2 MeOH, 1/2 BABB, let stand 10 minutes
9. Clear in 100% BABB, allow at least 20 minutes for this step, store at 4 degrees
10. Image embryo on confocal. To do this, embryo must remain submerged in BAB in a slide with a well. The optical surface (closest to the objective) needs to be a coverslip.

\*\*\* Note on clearing: You could probably skip the postfix step. Others in my lab don't do this because it was not part of the original protocol, and they have not had problems without the postfix step.

### **Solutions**

**Dent's Fix:** 1 part DMSO : 4 parts MeOH --> Fine to make up a big batch and store

**Dent's Bleach:** 1 part H<sub>2</sub>O<sub>2</sub> : 2 parts Dent's Fix --> Make up fresh

**BABB:** 1 part benzyl alcohol : 2 parts benzyl benzoate

Be careful with this solution. It eats through plastic.

**Blocking solution:** 5% serum (goat or donkey), 75% 1x PBS, 20% DMSO