**IHC protocol anti-MMP13 (rabbit polyclonal ab39012-abcam) (Eva Liu)**

**Day 1**
Paraffin Sections
1. Dry slides Over Night in 37C Hand heat paraffin for 1hr at 55C to melt wax
2. Deparaffinize slides in 2 changes of xylene x 5min each
3. Rehydrate in graded alcohol 100% ➔ 95% ➔ 70%, 3min each
4. PBS wash 5min
5. Antigen retrieval:
   - 10 min: sodium citrate buffer 95-97C (water bath=100), then cool 30 min
6. Wash PBS
7. Incubate slides in 3% H2O2 in methanol: 20min
8. PBS Wash 5 min
9. Block slides 10% HIFBS/PBS 60 min before primary antibody
10. Primary AB 1:200 in 10%HIFBS/PBS 4C overnight

**Day 2**
10. TBST wash 3x 5min each
11. Secondary AB (1:400 anti-rabbit HRP) in 10%HIFBS/PBS 1HR RT
12. TBST wash 3x 5min
13. DAB Detection
   1. 5ml ddH2O
   2. 2 drops buffer ➔ VORTEX
   3. 4 drops DAB ➔ VORTEX
   4. 2 drops H2O2 ➔ VORTEX
20. Stop reaction in ddH2O
21. Counterstain with Fastgreen 1 min ➔ Acetic Acid 30 sec ➔ ddH2O
22. Dehydrate in graded alcohol 70% ➔ 95% ➔ 100% ➔ Xylene
23. Coverslip