Primary Ab MMP13 abcam (ab39012) rabbit 1:50 (1:75, 1:100 works also!)
Negative Control: rabbit IgG ab172730 1.2ul in 100ul
**all instructions are done in standard staining dish unless otherwise noted*
**samples should be fully covered w/ solution during all incubations in humid chamber*

Day 1: ~3hrs

1) warm water bath to 60°C
2) Dewax & rehydrate slides
    a. 63°C 5 mins
    b. Citra-Solv wash 5 mins (3X)
    c. 100% EtOH wash 12 slow dips (2X)
    d. 95% EtOH wash 12 slow dips (2X)
    e. 70% EtOH wash 12 slow dips
    f. ddH2O wash 12 dips or 3 mins (2X)
3) **Unitrieve** 60°C 30 mins in *coplin jar*
4) rinse in water 3 mins (2X)
5) fresh **3% H2O2** incubate RT 10 minutes in *coplin jar*
6) rinse in water 3 mins (2X)

7) block w/ *Innovex Kit* **Fc-block** RT for 45 mins (*in humid chamber*)
8) rinse in ddH2O 2 quick change
9) block w/ *Innovex Kit* **Background Buster** RT for 45 mins (*in humid chamber)*
10) rinse in ddH2O 2 quick change
11) Primary AB in PBS (~50-70ul per sample) RT for 1hr or O/N 4°C (for controls- use PBS) (*in humid chamber)*

Day 2: ~2hrs

1) PBS wash 5-10 mins (3X)
2) Incubate w/ *Innovex Kit* **Secondary Linking Ab** at RT for 10 mins (*in humid chamber)*
3) PBS wash 5-10 mins (3X)
4) Incubate w/ *Innovex Kit* **HRP-enzyme** incubate RT for 10 mins (*in humid chamber)*
5) PBS wash 5-10 mins (3X)
6) fresh **DAB working solution** incubate RT for 5 mins (*in humid chamber)*
7) Rinse in PBS 3 quick washes
8) Mount with **Advantage Mounting Media**

Unitrieve (Innovex Biosciences- NB325)
Innovex Animal IHC kit (Innovex Biosciences- 329ANK)
-kit includes: Fc-block, Background Buster, Secondary Linking Ab, HRP-enzyme, DAB substrate buffer, DAB
Advantage Mounting Media (Innovex Biosciences- NB300)

DAB working solution: (time sensitive)
1 ml DAB substrate buffer
1 drop DAB

3% hydrogen peroxide (H2O2) (50ml):
1.5 ml H2O2 (30% stock solution)
48.5 ml H2O

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