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) 3% hydrogen peroxide (H2O2) (50ml):
1 ml DAB substrate buffer 1.5 ml H2O2 (30% stock solution)
1 drop DAB 48.5 ml H2O

References: