Cleaved Caspase 9 Protocol

1) Heat slides in 58 degrees for 1 hour
2) Xylene 5 min x 2
3) Rehydrate slides (100% alcohol x 3 min → 95% alcohol x 3 min → 70% alcohol x 3 min
4) PBS wash 3 min

- Antigen Retrieval

20 minutes in sodium citrate buffer pH 6 in steamer (97 degrees), then cool 30 min at room temp
Sodium Citrate buffer: 2.05 mL of 1 M sodium citrate, 450 uL of 1 M citric acid in 250 mL total volume (water that is microwaved for 1.5 minutes)

5) Wash PBS 3 min
6) 3% H_2O_2 in methanol 20 minutes (25 mL 30% H_2O_2 in 225 mL Methanol)
7) Wash PBS 3 min
8) Block slides in TNB (100 uL) 30 minutes
9) Primary antibody (Cleaved caspase 9 AB Cam 1:100 ab52298) diluted in TNB 100 uL/slide overnight at 4 degrees
10) Wash TBST 3 x 5 min each
11) Secondary antibody rabbit IgG biotinylated 1:400 in TNB, 1 hour Room temp
12) Wash TBST 3x 5 min
13) SA-HRP 1:100 in TNB, 100 uL per slide – 30 min room temp
14) Wash TBST 3x 5 min
15) Apply 100 uL Biotinyl Tyramide for 6 min (1:50 with 1x amplification diluent, can use 50-60uL if not enough stock)
16) Wash 3x 5min TBST
17) SA-HRP 1:100 in TNB, 100 uL per slide – 1 hour room temp
18) Wash 3x 5min TBST

- DAB development (5 mL H_2O with 4 drops Dab, 2 drops buffer, 2 drops H_2O_2)

19) Fast Green Stain (FG for 1 minute → 1% acetic acid 30 sec → dip H_2O)
20) Dehydrate (2 min each of 70% alcohol → 95% alcohol → 100% alcohol → xylene)
21) Coverslip