**Protocol for staining motile and primary cilia in formalin-fixed tissue:**

2X5 minutes Xylenes

1X3 minutes 100-95-95-95% ethanol

2X5 minutes 1XTBST

10 minutes at RT in 3% aqueous H2O2

2X5 minutes 1XTBST

**☐ Antigen retrieval:**

1. Antigen retrieval **buffer** is a 1:20 dilution (in water) of 20X EDTA solution (pH 8; Life Technologies, cat number 005500; 100 ml)
2. Place slides in 250 ml of buffer and subject to heating (I use a pressure cooker, 5 minutes at pressure with slow release). After pressure is released, leave slides in hot buffer (in the pressure cooker) for 10 minutes, then cool to RT in running diH2O.
3. Wash once in 1XTBST for 5 minutes

**☐ Block** 1 h at RT in **blocking diluent** (1%BSA, 1% milk, 10% normal donkey serum in 1XTBST)

**☐ Primary antibody (1:1000 in blocking diluent)** incubation for 1 hour overnight at 4oC or at room temp. Do not wash slides after blocking step, just tap off excess blocking solution

* **Antibodies:** Mouse anti-acetylated α-tubulin (Sigma, cat number T6793-.2ML), which stains the axoneme or stalk of the cilia. Rabbit anti-Gamma-tubulin (Sigma, cat number T5192-.2ML) stains the centrosome or basal body of the primary cilia (don’t really need gamma tubulin for staining motile cilia, just need acetylated tubulin).
* Acetylated tubulin 1:1000
* Gamma-tubulin 1:800

**☐** Wash 2 X 5 minutes 1XTBST

**☐ Incubate with secondary antibodies** for 1 hour at RT in the dark

 **Donkey anti-mouse-488** or 594 at **1:1000** for acetylated α-tubulin

 Donkey anti-rabbit-488 or 594 at 1:800 for gamma-tubulin (if used)

 Prepare in blocking diluent

**☐** Wash 2X5 minutes in 1XTBST

**☐ Apply CuSO4** solution: 10 mM CuSO4 + 5 mM ammonium acetate, pH 5

**Note** that this step is not necessary for detection of motile cilia, but it may help suppress autofluorescence. So this step can be skipped if autofluoresence isn’t a problem. I’m still working this out, it doesn’t hurt anything and helps with primary cilia detection).

**☐** Leave solution on for 30 minutes (upto 1 h) but use 30-min protocol

**☐** Wash 2 X 10 minutes in 1XPBS (washing time may have to increase if precipitates form under the coverslip over time, assuming that the problem is that CuSO4 reacts with the mounting media in some way). Could also wash with 1XTBS, but I’ve switched to PBS for this step with no problem.

**☐** Coverslip with Prolong Gold + DAPI + Seal with Nail polished around cover slip