**Anti-β-Catenin (#610154, BD Transduction Laboratories)**

**Paraffin/ formalin sections**

1. Deparaffinize:
	1. Xylene 2 x 5 min
	2. 100% EtOH 2 x 3 min
	3. 95% EtOH 2 x 3 min
	4. Automation buffer 1 x 5 min
2. Heat-Induced Epitope Retrieval Using the Decloaker. Fill with 1x citrate buffer (250 mL in a canister).
	1. Place in decloaker with 500 mL of dH2O, also put in the dH2O (250 mL each) in the other two empty canisters to make sure everything heats up evenly.
	2. Insert blank slides into empty slots in the rack to ensure even heating of slides.
	3. Set at **110°C for 15 min**. Let slides cool for an extra **10 min in the decloaker** when cycle is done.
	4. Let the slide **cool for extra 10 min** outside the decloaker 🡪 Rinse in **running dH2O for 3 min**
3. Block endogenous peroxidase with **3% H2O2** (5 mL 30% H2O2 + 45 mL MQ H2O) for **15 min**.
4. Rinse **3 x 5** min in **PBST (PBS + 0.1% TritonX-100)**
5. Block with **3% BSA** + **5% Normal Horse Serum in PBST** for **1 h RT**

= 30 mg BSA + 50 µL NHS + 950 µL PBST to make 1 mL blocking solution

1. Dilute **1:200, 1:400 Anti-β-catenin (#610154, BD)** in **3%BSA in PBST** @ **4°C O/N or 1h RT**
	1. 60 μL/section is sufficient – so that we don’t have to use much of 1° Ab
2. Rinse **3 x 5** min in **PBST**
3. Drain slides and apply **biotinylated horse anti-mouse** (**1:500**) in **3%BSA in PBST**, incubate 30 min RT.

1. Rinse **3 x 5** min in **PBST**
2. Apply Vectastain R.T.U. Elite (Vector Laboratories, PK7100) 30 min RT.
3. Rinse **3 x 5** min in **PBST**

1. Apply the **DAB** Chromogen (Dako). Incubate for **2 min RT**, wash with dH2O
2. Transfer slides to a dish and rinse in **running water for 3 min**.
3. Counterstain **30 seconds** in **hematoxylin**; rinse slides in tap water until water is clear
4. Gently agitate slides in **Automation Buffer** until the tissues turn blue (I did **30 sec**).
5. Dehydrate
	1. 95% EtOH 1 x 3 min
	2. 100% EtOH 3 x 3 min
	3. Xylene 2 x 5 min
	4. Coverslip with Permount