Immunohistochemistry for non-dissected fly tissues (appendages, etc.)

Day 1
1. Place body parts in 1 mL of 3-10% PBT for 30 min (for large enclosed section, like abdomens or pupae, piercing one or more holes or briefly washing samples in hexane will aid the process).
2. Remove PBT. Add 1 mL of 4% formaldehyde in 3% PBT. Leave at RT for 30 min.
3. Quick 2x wash in 3% PBT.
4. Rinse 3x for 20 minutes in 3% PBT at 4°C on nutator.
5. Remove PBT, add 1 mL superblock blocking buffer. Place on shakes for 1 hr at room temperature.
6. Remove blocking buffer, add primary antibodies in blocking buffer.

Day 3:
1. Quick 2x wash in 3% PBT.
2. Rinse 3x for 20 minutes in 3% PBT at 4°C on nutator.
3. Remove PBT; add secondary antibodies (1:1000) in blocking buffer. Make sure you’re using the correct secondary based on the species of the primary antibody (keep samples in continual darkness from this point onwards). If detecting more than one antigen, make sure the secondary antibodies are labelled with different fluorophores (AlexaFluor 488 and 568 work well together).

Day 5:
1. Quick 2x wash in 3% PBT.
2. Rinse 3x for 20 minutes in 3% PBT at 4°C on nutator.
3. Quick 2x wash in PBS.
4. Place in PBS for 20 hours at 4°C on nutator.
5. Transfer parts to mounting slides.
6. Carefully remove any PBS, and replace by mounting media.
7. Place coverslip and seal with clear nail polish (store at 4°C in dark until imaging).

Buffers:
1. 3% PBT buffer: Dissolve 3% (v/v) Tween 20 in 1X PBS (e.g. 3ml T20 in 97ml ddw).
2. Fixative (per 10ml): 4ml 16% Paraformaldehyde (new ampulla) + 1ml 10xPBS + 5ml ddw. Always mix fresh and keep at room temp.

The Hard DAPI mounting media does not work particularly well using this preparation method. Never leave samples overnight in 3% PBT.