**Steps for Immunohistochemistry on paraffin sections**

De-Paraffinization steps are done in the FUME HOOD

1. Make sure all solutions are filled to 250 ml mark
2. Sign up to use the set up because
3. all solutions are changed after 250 slides have been de-paraffinized and rehydrated

MATERIALS:

1. Xylenes: Fisher catalog number
2. Alcohol: Fisher catalog number A962F
3. Bovine Serum Albumin: Sigma catalog number A1503-506
4. Avidin Biotin Blocking kit: Vector Catalog Number SP2001
5. 30% Hydrogen Peroxide: Fisher Catalog number H325-100, can be used for one month

WASHING BUFFER: Make 6- 8 liters of Washing buffer

NOTE: If using HRP to detect binding of first reagent, use Phosphate Buffered Saline

If using Alkaline Phophatase to detect binding of first reagent, use Tris Buffered Saline

DILUTING BUFFER: this is 1% Bovine Serum Albumin dissolved in Washing buffer

1. Measure out 48 milliliters of washing buffer in a 50 ml tube
2. After taring the weighing scale using weigh paper, weigh out 500 milligrams of bovine serum albumin
3. Add the 500 milligrams of bovine serum albumin to the 48 millliliters and mix well by inverting a few times.
4. Return unused portion to the freezer to be thawed for the next time of use
5. Diluting buffer is used to dilute all reagents to be used in immunohistochemistry assays. Use antibodies at concentrations between 1-10 micrograms per milliliter.
6. Note: You will need 200 microliters on every slide

A. DE-WAX—Remove paraffin wax:

In the fume hood (Xylene is injurious if inhaled) place slides in slide holder in green tub with 250 ml of Xylene in the fume hood

1. FIRST XYLENE: Move slides holder up and down to ensure even coating sections with reagent
2. Leave slides in xylene for a minimum of 10 minutes or overnight, in the fume hood
3. Move slides to SECOND rack of xylene, move rack up and down to coat evenly and leave slides in the xylene for 10 minutes
4. Move slides to THIRD rack of xylene, move rack up and down to coat evenly and leave slides in the xylene for 10 minutes

B. RE-HYDRATE—can do on the bench:

1. FIRST 100% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent
2. SECOND 100% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent
3. FIRST 95% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent
4. SECOND 95% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent
5. FIRST 70% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent
6. SECOND 70% alcohol: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for 5 minutes in reagent

C. BUFFER WASH: Move slides holder up and down to ensure even coating of sections with reagent. Leave slides for a minimum of 5 minutes in reagent.

D. BLOCK ENDOGENOUS PEROXIDASES: All tissue have blood vessels and ALL blood vessels have red blood cells with endogenous peroxidases which must be removed ---If using HRP enzyme as the label to detect binding of first antibody.

1. Place 2.5 milliliters of 30% H2O2 in 250 milliliters of buffer
2. Place slides in this 0.3% H2O2 for30 minutes at room temperature
3. Followed by washing in three changes of wash buffer

E. BLOCK ENDOGENOUS BIOTIN:
Many tissues such as heart, liver, kidney, pancreas contain endogenous biotin which must be blocked—if using biotinylated reagents in the assay

Avidin Biotin Kit Vector Catalog SP2001 contains pre-made 0.1% Avidin in a white capped bottle and pre-made 0.01% Biotin in a black capped bottle

1. Overlay sections on the slides with 0.1% Avidin – 15 min.
2. followed by buffer rinses – 3x
3. Overlay sections with 0.01% Biotin – 15 min.
4. followed by buffer rinses – 3x

F. MAKE THE HUMIDIFIED CHAMBER:

Pour 250 milliliters of the washing buffer in to the lower container of the staining tray.

Once the lid is placed on top this then becomes the humid chamber which is used for all incubation steps

G. BLOCK ENDOGENOUS NON-SPECIFIC COLLAGEN BINDING:

Overlay sections on the slides with diluting buffer (1% BSA/PBS) for a minimum of 5 minutes while making dilutions of the primary reagent.

**IF ANTIGEN RETRIEVAL IS NEEDED—PROCEED AS RECOMMENDED AT THIS STEP, BEFORE OVERLAYING PRIMARY REAGENT**

H. PRIMARY REAGENT: Overlay sections with 200 microliters of primary reagent,

---Each diluted to between 1 and 10 micrograms per milliliter.

In every assay:

Always include a slide, which will receive a positive control reagent

And always include a slide, which will receive a negative control reagent

If incubations are going overnight, cut parafilm to fit over the slides and overlay so that the sections do not dry out during the overnight incubation.