abcam

Tissue processing protocol

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Overview

Once the tissue is fixed, it needs to be processed so that the soft tissue is adequately supported for cutting in to thin sections of up to 5µm thickness. The tissue is dehydrated, cleared and then infiltrated with medium to enable sectioning. Paraffin wax is the most common medium used for immunostaining.

Paraffin Tissue processing

1. After fixation, rinse tissue with PBS until fixative is completely removed.

2. Dehydrate tissue using ethanol in the following sequence

Solution	Incubation time
50% Ethanol	10 min
70% Ethanol	10 min
80% Ethanol	10 min
95% Ethanol	10 min
100% Ethanol	10 min
100% Ethanol	10 min
100% Ethanol	10 min

3. Exchange ethanol with xylene in the following sequence

Solution	Incubation time
2:1 Ethanol: Xylene	10-15 min
1:1 Ethanol: Xylene	10-15 min
1:2 Ethanol: Xylene	10-15 min
100% Xylene	10-15 min
100% Xylene	10-15 min
100% Xylene	10-15 min

4. Exchange xylene with paraffin. The following steps are done in a vacuum oven set at 54-58°C. Paraplast X-tra or Paraplast Plus (the Plus has DMSO added to facilitate infiltration) can be used. Do not let the paraffin exceed 60°C for prolong periods of time because this will degrade the paraffin polymers and make it hard and brittle.

Solution	Incubation time
2:1 Xylene: Paraffin	30 min
1:1 Xylene: Paraffin	30 min
1:2 Xylene: Paraffin	30 min
100% Paraffin	1-2 hr
100% Paraffin	1-2 hr or Overnight

5. Embed in fresh new paraffin and orient tissue as desired before it hardens (vertical for embryos).

Frozen tissue embedding

1. Tissue ready for processing should be fixed and in stored in PBS.

2. Sucrose infiltration.

Solution	Incubation Time
10% Sucrose	15 min or until sample drops to bottom of vial
2:1 10% Sucrose: 30% Sucrose	15 min or until sample drops to bottom of vial
1:1 10% Sucrose: 30% Sucrose	15 min or until sample drops to bottom of vial
1:2 10% Sucrose: 30% Sucrose	15 min or until sample drops to bottom of vial
30% Sucrose	15 min or until sample drops to bottom of vial
30% Sucrose	15 min or until sample drops to bottom of vial
30% Sucrose	15 min or until sample drops to bottom of vial

3. Partially fill dry ice container with dry ice and add methanol to create a cool bath, let sit.

4. Label Tissue Tek wells with each animal number and fill with OTC (TissueTek) freezing compound.

5. Remove excess sucrose from tissue by blotting on Kimwipes and place tissue in center of well filled with OTC.

6. Orient tissue into the bottom of the well and freeze by floating on methanol bath. CAUTION: do not get methanol on the OTC, it will not freeze correctly.

7. Place frozen tissue blocks in -20°C freezer after they are frozen.

8. The tissue blocks are ready to be sliced after they are frozen completely. Do not store slides in the cryostat over night, they will dry out and be no good. It is also a good idea to place all tissue into plastic bags in the -20 frost free freezer to reduce drying out during storage.

9. Slice sections on the cryostat.

Glycol methacrylate (GMA) Embedding

Advantages of using GMA

- Water miscible, doesn't require dehydration and rehydration steps.
- No need to eliminate resin before staining.
- Low viscosity, penetrates tissue easily.
- No crosslinking, no antigen retrieval.
- Good antigen presentation.
- Good morphology preservation (cellular localisation).
- Low temperature processing.
- Can cut to very thin sections (1-2 µm) making the most of very small biopsies – very good resolution

Fixation

Several methods of tissue fixation can be used for GMA.

Fixing in acetone usually gives good results. Use the following method:

1. Place biopsy immediately in ice cold acetone containing protease inhibitors.
2. Fix overnight -20°C.
3. Replace fixative with acetone (room temperature) 15 min.

Processing

1. Place biopsy in methyl benzoate for 15 min (This helps infiltration of GMA into the tissue).
2. Place biopsy in 5% methyl benzoate in GMA 4°C. 3 times for 2 hr.

Embedding

Follow the kit manufacturer's instructions for embedding into GMA itself. The GMA will need to be polymerised using a catalyst (provided in commercially available kits) and left to set for 48 hr at 4°C.