TRAP Staining for Formalin-fixed Paraffin Embedded Sections

Procedure

1. Pre-warm MQ H_2O (~50ml per coplin jar) at 37-42°C (for step 3b) ~30min.

2. Deparaffinize slides ~60min:

   Best if you can heat, but doesn't really matter

   Note – never let your slides dry out!!!!!! Throughout this whole procedure

   Deparaffinize
   - 1.) Histoclear x 10min
   - 2.) Histoclear x10min

   Rehydrate
   - 3.) 100% EtOH (or Flex 100) x 3 min
   - 4.) 100% EtOH (or Flex 100) x 3 min
   - 5.) 95% EtOH (or Flex 95) x 3 min
   - 6.) 95% EtOH (or Flex 95) x 3 min
   - 7.) 70% EtOH x 5min
   - 8.) MQ H_2O x 5 min

   Note all of the above steps do not have to be exact… you can leave for longer

3. Prepare the staining solution (according to Beaker B in steps 3 and 4 of the Acid Phosphatase, Leukocyte Kit).

   a. Make 1 mL Diazotized Fast Garnet GBC Solution (1:1 Fast Garnet GBC Base Solution: Sodium Nitrite Solution). Mix by gentle inversion, let stand for 2 min

   b. Mix the following solutions while stirring (following volumes = 1 coplin jar):

   MQ H_2O pre-warmed to 37°C – **45mL**
   Diazotized Fast Garnet GBC Solution (6a) – **1mL**
   Naphthol AS-BI Phosphate Solution – **0.5mL**
   Acetate Solution – **2mL**
   Tartrate Solution – **1mL**

   ***scale volumes as necessary for container**

   c. Pour solution into coplin jar and ensure that temperature is **37-42°C** (Important!)

4. Incubate slides at 37-42°C in staining solution for 1 hour.

5. Rinse slides thoroughly in MQ H_2O (2x 5-10 min).

6. Counterstain with Hematoxylin Solution, Gill No.3 for 2 min.

   Put in warm tap water bucket and run warm tap water over it for 8 min

   Coverslip with Fluoromount G (Aqueous mounting medium)

   Use about 100ul permount per slide
   Wipe off excess
   Seal with nail polish
   Let dry at least 1 hour before looking at on scope (usually O/N)