

TMB METHOD FOR LM

Solution A:

200mg Sodium Nitroferricyanide (0.2g top balance)
185ml dist waster
10ml Sodium Acetate buffer pH 3.3

Solution B:

10mg TMB (0.01gm) (in left hand freezer)
5ml absolute alcohol
---- heat to 37^oC. Use thermometer, do not overheat

Solution C:

950ml dist water
50ml sodium acetate buffer pH3.3

1. Wash tissue in 2 changes dist water 2mins each
2. Mix solutions A and B and immediately place tissue in this solution. (Keep this solution in ice bath and cover with tin foil.)
3. **After 5 mins add 60µl hydrogen peroxide** (in right hand fridge)
4. Incubate for 10-20 minutes and monitor for build up of contaminate in solution.
5. 5 X 2min rinses in solution C.
6. Mount sections

7. When sections are dry coverslip as follows:-
 - a. 70% alcohol 20secs
 - b. 100% alcohol 20secs
 - c. Xylene 5mins
 - d. Coverslip. **DO NOT PUT ON HOT PLATE**

DO NOT EXPOSE TO ALCOHOL

DO NOT EXPOSE TO HEAT

SODIUM ACETATE BUFFER, pH3.3 FOR TMB

200 ml 1.0M Sodium Acetate (27.2 g in 200ml dH₂O)
200ml dH₂O
add 190ml 1.0M HCl
add dH₂O to 1000ml
Adjust pH to 3.3 with NaOH or Acetic Acid

NEUTRAL RED STAIN FOR TMB

Add 40ml of pH 4.8 acetate buffer (500 ml 0.1N acetic acid, 750ml 0.1N sodium acetate) to 1 lt. aqueous neutral red solution.
Filter before using.