TMB-TUNGSTATE METHOD FROM IDA LLEWELLYN-SMITH

1. Incubate tissue in following solution for 10 mins.

   10ml 0.1M Phosphate buffer pH 6.0. (see note 1)
   0.5ml 1% Ammonium p-tungstate (see note 2)
   0.25 ml 0.2% TMB in absolute alcohol (see note 3)

2. Add 10μl 3% H_2O_2 and let tissue react for up to 1 hour. (see note 4)

3. Wash in 0.1M phosphate pH 6.0 (2 x 5mins)

This TMB reaction product is stable in everything except osmium so you can mount some sections for LM, dehydrate as normal and coverslip.

TO STABILIZE FOR EM

1. Incubate tissue in following solution for 10 mins

   10ml 0.1M Phosphate buffer pH 6.0.
   200μl 1% Cobalt Chloride
   200μl 25 mg/ml DAB in water
   200μl 0.3% H_2O_2

2. Wash in 0.1M phosphate pH 6.0 (3 x 5mins)
3. 1% Osmium Tetroxide in phosphate pH 6.0 (1 hour)
4. Rest of procedure as normal

NOTES

1. Dilute stock 0.2M buffer with H_2O and add HCl to bring to ph 6.0
2. Takes a long time to dissolve (>30 mins), Use magnetic stirrer. Can be stored at **room temperature** for about 1 week.
3. This is 0.01g TMB in 5ml alcohol. (According to protocol this solution can be stored for 2 months at 4°C.)
4 3% H_2O_2 = 1ml H_2O_2 in 10ml dist H_2O_2. (stockH_2O_2 = 30%)