

TPBS=Tris phosphate buffered saline = 0.05M PBS + 0.01M TRIS

NHS=Normal horse serum. (Can substitute normal goat serum)

Cut Sections in PBS

Permeabilize immediately in 50% ETOH. 45 min.

Wash 0.1M PO₄, pH 7.4. and swirl. 10 mins. 2 Washes

Block: 10% Normal Horse serum in TPBS 1Hr

NO WASH

Incubate: *ExtrAvidin-HRP 1:1500 and 1%NHS in TPBS. 1-2 days in cold room. Can Use Elite ABC kit.

Make up in TPBS.

Day 2

Wash 3 x 15 min. in TPBS

Reaction: TMB-tungstate-GOD

Note: 1) this is for cell: would need higher conc. TMB for back-fill.

Reaction mix: Soln A.

20 ml 0.1M PO₄ buffer pH6

1.0ml 1% ammonium paratungstate (make weekly, store RT)

0.25 ml 0.2% TMB (free-base) in abs. ETOH

0.2 ml 20% D-glucose + 0.05%

0.2 ml 0.4% NH₄Cl

Pre-incubate for 12 minutes using 1 ml per well

Add another 1ml of solution A containing 2µl GOD for 5-10 minutes

Wash 3x10 mins in PO₄ buffer **pH 6.0**. (V. Important)

Dissolve 10mg DAB tablet in 10 ml d H₂O

Add 10ml 0.2M PO₄, pH 6.0

Add 200µl 0.4% NH₄Cl

400µl CoCl₂ (dropwise)

Add 200 µl 20% d-glucose

FILTER

Add 20µl Glucose oxidase

(keep glucose oxidase over ice water)

Reaction should go in 10-20 min

Wash 0.1M PO₄, pH 6.0. 3 x 10 min

EM Process

Osmicate at 6.0.