

Cholera Toxin - B Immunocytochemistry

ALL AT ROOM TEMP. USE SHAKER TABLE

1. Wash one time with TPBS.
2. Incubate in 50% ethanol (ddH₂O) for 10 minutes.
3. Wash four times with TPBS mix (1% normal **horse** serum, 0.3% triton) 5 min. each.
4. Incubate for one hour TPBS special mix (**10%** NHS, 0.3% triton) - "blocking solution"
5. Take out block. Add 400 ul of CT-B antibody (raised in goat, MSRS antibodies, prod# 703) 1:20,000 (in TPBS mix) 24-36 hours. Mix antibody into solution gently before applying.
6. Wash three times with TPBS mix, 5 min each.
7. Secondary antibody - donkey anti sheep (Sigma B-7390) 0.2% (20 ul in 10 ml TPBS mix) - at least one hour or overnight. (400 ul per well) Mix antibody into solution gently before applying.
8. Wash three times with TPBS plus 0.3% triton, 5 min each.
9. Incubate 90 minutes with 0.1% ExtrAvidin E-2886 (10 ul in 10 ml TPBS + triton)
10. Wash three times with TPBS only.
11. On ice, dissolve 1 DAB tablet into 18.8 mls TRIS. Vortex. Slowly stir, drip in the following:
200 ul of 0.4% NH₄Cl (in ddH₂O)
200 ul 20% β-D glucose (in ddH₂O)
Last - 800 ul of 1% Nickel Ammonium Sulfate (ddH₂O). Vortex.
12. Filter through Whatman #113 filter paper. After two folds, tear wide top off so that filter is miniature. (Otherwise, too much DAB will be absorbed by filter)
13. Remove TPBS and put 400 ul of DAB solution into each well for ten minutes.
14. Take 10ml of DAB solution and add 10ul glucose oxidase - vortex.
15. Add 400 ul to the wells with DAB **still there**, until reacted "enough" (4 to 30 min)
16. Take off DAB solution into DAB waste container. Replace with TPBS.
17. Wash with TPBS three times.

NOTE: DAB is toxic if swallowed or inhaled. Please use gloves when manipulating.
ALSO: Handle DAB under the hood.