

O/E-1 and GAP4-43 FAB Fragment IHC

- Use unconjugated Fab fragment to convert first primary antibody to a different species as both primary antibodies are host species rabbit.

1) Bake slides at 42°C for 2 hours

Day 1

2) Wash slides in PBS 2 x for 10 minutes each

3) Incubate in 0.3% PBST, 0.3% H₂O₂ for 20 minutes

4) Wash slides in PBS 2 x for 10 minutes each

5) Circle with PAP pen

6) Block in 10% NDS for one hour

7) Incubate with first primary antibody (O/E-1, 1:200 in 0.3% PBST) overnight at 4°C

Day 2

8) Wash slides in 0.3% PBST 3 x for 10 minutes each

9) Incubate in unconjugated FAB fragment goat-anti-rabbit (1:50 in 10% NDS with 1:66.67 10% NDS) for one hour

10) Wash slides in 0.3% PBST 3 x for 10 minutes each

11) Wash slides in PBS for 10 minutes

12) Incubate with second primary antibody (GAP-43 1:500 in 10% NDS) overnight at 4°C

Day 3

13) Incubate with both conjugated secondary antibodies for one hour in PBS with 1:66.67 10% NDS

 Cy3 donkey-anti-goat 1:500

 FITC donkey anti-rabbit 1: 50

14) Wash slides in PBS 2 x for 10 minutes each

15) Coverslip with Mowiol