Immunostaining Protocol

Staining is done with free-floating technique (not on slides). All washes and incubations are done at room temperature on shaker. DO NOT LET SECTIONS DRY UNTIL MOUNTING STEP.

ONE DAY PROTOCOL

1. Wash with 1X PBS, 3 x 5 min
2. Block with 10% normal goat serum (NGS) + 0.5% Triton-X in 1X PBS for 30 mins
   –for 12-well plate size: 1mL solution per well
   –to make 10mL solution: 1mL NGS + 0.5mL 10% Triton-X
   –to make 10% Triton-X stock: for 100mL: 10 mL Triton-X, 90 mL 1X PBS
3. Incubate with antibodies (diluted antibodies except lectin 1:1000 in 5% NGS + 0.25% Triton in 1X PBS), 4 hours
   –for 12-well plate size: 1 mL per well
   –**for Lectin-biotin: use 1:500 dilution**
4. Wash with 1X PBS, 3 x 5 min
5. Incubate with 2° antibodies (diluted in 1:1000 in 5% NGS + 0.25% Triton in 1X PBS), 2 hours (**Protect from light!)**
6. Wash sections with 1X PBS for 2 x 5 min (**Protect from light!)**
7. Incubate with DAPI (1 uL of 5mg/mL in 1mL PBS) for 5 mins (**Protect from light!)**
8. Wash sections with 1X PBS for 2 x 5 min, mount onto slide (**Protect from light!)**
9. Allow sections to air dry overnight before coverslip with Fluoromount-G (**Protect from light!)**

TWO DAY PROTOCOL

Day 1: After Sectioning

1. Wash with 1X PBS, 3 x 5 min
2. Block with 10% normal goat serum + 0.5% Triton-X in 1X PBS (10mL: 1mL NGS +0.5 mL 10% Triton-X), 3 hours
   –10% Triton-X: for 100mL: 10mL Triton-X, 90 mL 1X PBS
3. Incubate sections with antibodies (diluted in 1:1000 in 5% normal goat serum + 0.25% Triton in 1X PBS) overnight
   –for Lectin-biotin: use 1:500 dilution; MECP2: 1:1000
   –stored remaining blocking solution at 4°C

Day 2:

1. Wash sections with 1X PBS, 3 x 5 min **(before washing, remove diluted blocking solution from refrigerator)**
2. Incubate sections with fluorescent conjugated secondary antibodies (diluted in 1:1000 in 5% normal goat serum + 0.25% Triton in 1X PBS) for 4 hours (**Protect from light!)**
3. Wash sections with 1X PBS for 2 x 5 min
4. Incubate with DAPI for 5mins (**Protect from light!)**
   –1 uL of 5mg/mL in 1 mL PBS; make sure DAPI is dissolved completely by vortexing prior to application
5. Mount onto slide (**Protect from light!)**
6. Air dry overnight before coverslip with Fluoromount-G and seal with nail polish (**Protect from light!)**