Indirect Immunofluorescence (method) Protocol

If tissue is fresh (unfixed), fix with 4% PFA: 5min

Rinse slides in PBST: 3X10min

Blot slides- do not allow to dry
- tap firmly on paper towel

Apply blocking serum: 20min
- cover each slide with 1/2 piece of parafilm to evenly spread and protect from drying out
- place in dark, humidified chamber (room temp)

Blot slides- do not allow to dry

Apply primary antiserum to each slides (150µL/slides): Overnight
- cover each slides with 1/2 pieces of parafilm to evenly spread and protect from drying out
- incubate in dark, humidified chamber (10-16 hours at 4°C)

Bring slides to room temperature (in dark, humidified): 30min

Rinse slides in PBST: 3X10min

Apply blocking serum: 20min
- secondary antibody species should match block
- cover each slide with 1/2 piece of parafilm to evenly spread and protect from drying out
- place in dark, humidified chamber (room temp)

Blot slides- do not allow to dry
- tap firmly paper towel

Apply secondary antiserum to each slide (150µL/slide): 60min
- cover each slide with 1/2 piece of parafilm to evenly spread and protect from drying out
- incubate in dark, humidified chamber (room temp.)

Rinse slides in PBST: 3X5min

Blot slides - do not allow to dry
- tap firmly paper towel

Coverslip with Fluromount G
- put 3-4 drops on end of slide and lay down coverslip slowly from one end to the other; using thin needle to lower coverslip.

Allow slides to dry in a flat position (4°C): Overnight
- wipe dirty slides with ethanol to clean

Store slides at 4°C, -20°C, or -80°C