



# KIDDRC Histology Services Protocols

For more info contact: Jing Huang, Histology Specialist  
913-588-5996

## 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 (150 $\mu$ L/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 (150 $\mu$ L/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