

# Immunofluorescence Staining

Direct immunofluorescence method is used to detect the deposit of immunoglobulins, complement components, fibrinogen, etc. in tissues. This technique is usually performed on frozen sections. The primary antibody is conjugated to fluorescein binds directly with the antigen and can be detected by the fluorescent tag using a fluorescent microscope.

## Specimen

**Frozen tissue only.**

1. Fresh tissue (kidney, lung, skin, etc.) is frozen in -80°C freezer.
2. Embedding: OCT medium sectioning at 7µm.

## Controls

1. Use a frozen control of tonsils.

## Procedure

1. Place some OCT in plastic freezing boat.
2. Place tissue in OCT and orient according to standard embedding protocol.
3. Freeze in -80° Freezer.
4. Remove block from freezer and place in cryostat.
5. Cut sections from frozen block and place on coated slides. Sections should be cut at 7 microns on the cryostat.
6. Fix in cold acetone for 10 minutes.
7. Air dry.
8. Place slides in washing PBS for 5 minutes. OCT medium will dissolve in this wash.
9. Place antibodies on slides for 30 minutes. Slides must be kept in dark.
10. Rinse in PBS.
11. Coverslip with fluorescent mounting medium.
12. Store slides in cardboard folder in refrigerator until ready to screen.

**Note:** Using the Ventana automated procedure, slides will be placed on machine at Step 9 and resumed with Step. 10.