

## COLLAGEN VISUALIZATION BY INDIRECT IMMUNOFLUORESCENCE (1)

*Fresh 5µm thick cryosections should preferably be used in the following protocol.*

### **INDIRECT IMMUNOFLUORESCENCE TECHNIQUE**

**All steps should be performed at ± 20°C, in humid atmosphere**

1. Application of the primary specific antibody and control serum (non immune serum) diluted in PBS +3% BSA according to the recommended dilution (generally 1:40 and over) : 30min
2. Washing with PBS : 1 x 5min  
2 x 10min
3. Application of fluorescent immunoglobulins diluted in PSB +3% BSA according to the previously determined dilution (1:100 for fluorescein) : 30min
4. Washing with PBS (without or with 3 drops of Evans Blue solution) : 1 x 5min  
2 x 10min
5. Mounting in buffered glycerin.

### **REAGENTS**

- . Bovine Serum Albumin (BSA) (Sigma, A9647)
- . Buffered glycerin for IF (Biorad, 74921)
- . Evans Blue counterstain (Sigma, E0133)
- . Fluorescent anti-rabbit or mouse IgG (Biorad, 74561 or 74411 respectively)
- . Phosphate Buffered Saline (PBS), pH = 7.2-7.4 (bioMérieux, 75511)