

## COLLAGEN VISUALIZATION BY INDIRECT IMMUNOFLUORESCENCE (2)

**THIS PROTOCOL IS SPECIFICALLY ADAPTED TO THE IDENTIFICATION OF RAT TYPE V COLLAGEN AND OF THE TYPES II AND IX COLLAGEN OF ALL SPECIES.**

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

### **PRE-TREATMENT**

1. Tissue fixation in 2% paraformaldehyde at room temperature ( $\pm 20^{\circ}\text{C}$ ) : 30min
2. Treatment by hyaluronidase diluted at 0.2% in PBS + 3% BSA : 30min, **37°C**

Wash quickly first and then again for 15mn with PBS after each of the above 2 steps.

### **INDIRECT IMMUNOFLUORESCENCE TECHNIQUE**

**All steps should be performed at  $\pm 20^{\circ}\text{C}$ , 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) : overnight
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 rabbit or mouse IgG (Biorad, 74561 or 74411 respectively)
- . Hyaluronidase (Sigma, H3506 type I-S)
- . Paraformaldehyde (Sigma, P6148)
- . Phosphate Buffered Saline (PBS), pH = 7.2-7.4 (bioMérieux, 75511)