

PEROXIDASE IMMUNO-LABELLING TECHNIQUE FOR TISSUE LOCALIZATION OF COLLAGENS (1)

THE UNDERLINED STEPS IN THE FOLLOWING PROTOCOL ARE PARTICULARLY USEFUL IN THE CASE OF IMPORTANT BACKGROUND AND WITH RAT TISSUES.

Unless specified otherwise, all steps must be carried out at $\pm 20^{\circ}\text{C}$, in a wet chamber.

- **Fixing** in AFA (750ml Alcohol + 20ml Formalin + 50ml Acetic acid + 180ml distilled water) or in Bouin's liquid.
- **Embedding** in paraffin.
- **Section (5 μm thick) sticking** on silanized slides.

• **Pre-treatment / Deparaffinization ;**

- Xylene : 10min
- Xylene-95% alcohol (v/v) : 10min
- 95% alcohol : 10min
- 95% alcohol-distilled water (v/v) : 10min
- Cleansing with running tap water : 30min

• **Treatment ;**

- Glycin-HCl (100mM) / TBS* + NH_4Cl * (50mM) in TBS : 30min
- Hyaluronidase (Sigma : H3506 type I-S) at 0.5% in TBS : 60min, **20°C**

Rinse quickly first and then again for 5min with TBS after each of the above 2 steps.

• **Immuno-labelling ;**

- **Primary antibody*** diluted in TBS + 3% BSA* + **1% NGS*** : overnight
- **Inhibition of unspecific activity (TBS + 3% BSA + 0.9% NaCl)** : 20min

Rinse quickly first and then again for 10 min with TBS + 0.2% Tween 20 after each of the above 2 steps.

- **Inhibition of endogenous peroxidases** (10ml TBS + 3% BSA + 500 μl H_2O_2 at 30 vol. + **10mg NaN_3 ***) : 20min
- **Labeling** : Envision kit (Dako, K4002) : 45min

Rinse quickly first and then again for 10 min with TBS after each of the above 2 steps.

Staining ;

- DAB*+ chromogen (Dako, K3468) : 2-5min
(1.5ml TBS + 0.5ml kit buffer + 2 drops of DAB+)

Rinse for 10min with running tap water.

. Counter staining ;

- Aqueous Harris Hematoxylin (diluted 2/3 in distilled water) : 5min

Rinse for 10min with running tap water

- . Differentiation** with ammonia water (250ml water + 2ml ammonia at 30%) : 2min

- . Section Mounting** in aqueous mounting medium
(Faramount, Dako, S3025)

BSA* : Bovine Serum Albumin

DAB*: Diaminobenzidine

NaN₃* : Sodium azide

NGS* : Normal Goat Serum

NH₄Cl* : Ammonium Chloride

Primary antibody*: Specific, purified rabbit ECM antibody

TBS* : TRIS buffer , pH 7.6 (Dako, S3001)

(1) ; Collagens and other connective tissue proteins (elastin, fibronectin and laminin).