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IHC TECHNIQUE FOR THE LOCALIZATION OF COLLAGENS IN FROZEN TISSUE SECTIONS (1)

THE UNDERLINED STEPS OF THE FOLLOWING PROTOCOL ARE ESSENTIAL WITH RAT TISSUES AND IN CASES OF STRONG BACKGROUND.

Unless specified otherwise, all steps must be carried out in a humid chamber at ± 20°C.

. **Sticking** on silanized slides of 5µm thick frozen tissue sections.

. **Fixation** of tissue sections in ice cold acetone (-20°C) : 10min

. **Air drying** at room temperature (*± 20°C*) : 10min

. **Treatment ;**

- Hyaluronidase (Sigma : H3506 type I-S) 0.2% / TBS : 15min, **37°C**

Rinse quickly first and then again for 5min with TBS.

. **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 + 500µl H_2O_2 at 30 vol. + ***10mg NaN_3)**** : 20min
- **Labelling :** 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.

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. **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
NaN_3* : Sodium azide
NGS* : Normal Goat Serum
NH_4Cl* : Ammonium Chloride
Primary antibody*: Specific, purified rabbit ECM antibody
TBS* : TRIS buffer , pH 7.6 (Dako, S3001)