KARLAN ®

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ECM / COLLAGEN ANTIBODY TITRATION BY ELISA

This test is used to check on the specificity and titer of an antibody or to detect its possible cross-reactivity with different antigens.

REAGENTS AND EQUIPMENT

- 96 wells flexible assay microplates (Microtest III, Falcon, 3912)
- PBS, pH = 7.2-7.4 (BioMerieux, 75511)
- BSA (Sigma, A9647)
- 30% H_2O_2 (Sigma, H0904)
- 4 Aminoantipyrine (4 AMP, Sigma, A4382)
- Phenol (Sigma, P3653)
- Peroxidase-labelled rabbit and mouse anti-IgG (Biorad, 75011 and 75031 respectively)
- Chromogen solution :
  - PBS : 50ml
  - Phenol : 118mg
  - 4 AMP : 24mg
  - 30% H_2O_2 : 10µl (added extemporaneously)
- Microplates stirrer (Dynatech-Vari Shaker)
- Spectrophotometer (Titertek-Multiskan)

METHOD

1. Antigen fixation ;

- Dilute the antigen in PBS at the appropriate concentration
- Add 200 µl of this solution in each well
- store overnight at + 4°C
- Wash 3 times for 5 min. each in PBS (at + 20°C)

2. Incubation with the primary antibody ;

- Prepare a range of antibody dilutions in PBS + 3% BSA
- Add 100µl of each antibody dilution per well
- Incubate for 90 min. at + 20°C under moderate stirring
- Wash 3 times for 5 min. each in PBS (at + 20°C)

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3. <u>Incubation with the secondary antibody - peroxidase conjugate</u> ;

- Dilute the appropriate conjugate at 1:100 in PBS + 3% BSA
- Add 100µl of this solution per well
- Incubate for 90 min. at + 20°C under moderate stirring
- Wash 3 times for 5 min. each in PBS (at + 20°C)

4. <u>Colorimetric reaction</u> ;

- Add 100µl of the chromogen solution per well
- Incubate for 90 min. at + 20°C under moderate stirring

Read the optical density of the stained reaction with the spectrophotometer at 450ηm