KARLAN ®

KARLAN RESEARCH PRODUCTS CORPORATION
251 Jennifer Drive, Cottonwood,AZ 86326 USA
Tel: (928) 634-1492 Fax: (928) 634-1496
Email: info@karlan.com www.karlan.com

ECM / COLLAGEN ANTIBODY ELISA INHIBITION TEST

This particular test is used essentially to check on the specificity of an antibody.

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. Pre-incubation of the primary antibody with an excess of antigen ;

- The appropriate antibody dilution should be selected to correspond to the initial part of the steeper zone of the ELISA curve D_0=f (antibody dilution).
- Prepare a 2 times concentrated antibody solution by diluting the specific antibody in PBS + 3% BSA to twice the above appropriate antibody dilution.
- Mix 20µl of a 1mg/ml antigen solution and 50µl of the above antibody solution in a microtube ; complete to 100µl with 30µl of PBS + 3% BSA.
- Incubate overnight at +4°C.

KARLAN ®

KARLAN RESEARCH PRODUCTS CORPORATION
3343 Industrial Drive, Unit 9, Santa Rosa, CA 95403 USA
Tel: (707) 576-1225 Fax: (707) 576-1349
Email: info@karlan.com

3. Incubation with the primary antibody ;

- Transfer the content (100µl) of the microtube into one coated well of the microplate.
- Incubate for 90 min. at + 20°C under moderate stirring
- Wash 3 times for 5 min. each in PBS (at + 20°C)

4. Incubation with the secondary antibody - peroxidase conjugate ;

- 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)

5. Colorimetric reaction ;

- 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 450nm