Collagen ELISA Protocol

At least eleven genetically distinct gene products are collectively referred to as ‘collagen types’ or other proteins and proteoglycans of the extracellular matrix. In humans, collagens are composed of about twenty unique protein chains that undergo various types of post-translational modifications and are ultimately assembled into a triple helix. This results in great diversity between collagen types. Collagens are highly conserved throughout evolution and are characterized by an uninterrupted “Glycine-X-Y” triplet repeat that is a necessary part of the triple helical structure. For these reasons, it is often extremely difficult to generate antibodies to collagens. The development of type-specific antibodies most often is dependent on non-denatured three-dimensional epitopes.

Rockland extensively purifies collagens for immunization from human and bovine sources by limited pepsin digestion and selective salt precipitation. After isolation from rabbit antiserum, these antibodies are extensively cross-adsorbed by immunoaffinity purification to produce ‘type’ specific antibodies that are well suited to detect extracellular matrix proteins in normal and disease state tissues.

Procedure

1. Coat the plate with unlabeled anti-collagen (type specific) at 10 µg/mL.
2. Use biotinylated anti-collagen (same specificity) at a concentration of 0.1 µg/mL.
3. Dissolve the collagen firstly in 0.5 M Sodium Acetate.
4. Dilute the collagen in 0.05 M Tris Cl, 1.0 M Sodium Chloride, pH 7.4.
5. Perform serial dilutions of the collagen using the Tris/NaCl buffer.
6. Perform all steps at 4°C using overnight incubations.
7. All buffers are to be at 4°C.
8. Use Streptavidin-HRP for detection.