Sandwich ELISA Protocol for Type-Specific Collagen Antibodies

At least eleven (11) 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 (20) 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, generating type-specific antibodies to collagens presents unique challenges. It is an absolute requirement to use non-denatured three-dimensional epitopes to develop these type-specific antibodies. Rockland extensively purifies collagens for immunization from human and bovine sources by limited pepsin digestion and selective salt precipitation. This maintains the three-dimensional structure of the collagen.

After isolation from rabbit antiserum, these antibodies are repeatedly and exhaustively 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. For instance, anti-Collagen Type I is first purified against immobilized type I collagen and then is passed over immobilized type II collagen repeatedly until no additional antibody is bound. This process is applied successively for all other collagen types rendering the Collagen Type I antibody specific for collagen I with minimal cross-reactivity to all other collagen types.

Because Rockland collagen antibodies are designed with specificity to recognize only non-denatured three-dimensional epitopes, assays that maintain native collagen structure work best. For ELISA, capture (a.k.a. sandwich) ELISA is recommended. A “titering” ELISA, on the other hand, is not recommended because the native collagen structure is disrupted when collagen is directly bound to the ELISA plate. As a result, loss of detection specificity may occur due to: (1) incomplete binding to the plate for some collagen types, (2) partial denaturation of some collagens, and/or (3) partial or incomplete blocking.

This protocol accounts for the design and function of these collagen antibodies to optimize the specificity of the antibody. Deviations to the protocol are encouraged but may require additional optimization by the end-user to preserve antibody specificity.

I. Reagents Required

Reagent	Preparation
Blocking Reagent: Use BSA-50 and Tween-20	N/A
Capture Antibody: For capture of Collagen Type I, use Anti-Collagen Type I	N/A
Collagen Analytes: For capture of Collagen Type I, use Human Collagen Type I.	N/A
Detection Antibodies: For detection of Collagen Type I, use Anti-Collagen Type I Biotin Conjugate.	N/A
Detection Reagents: Use Streptavidin Peroxidase Conjugate and TMB ELISA Peroxidase Substrate	N/A

II. Procedure

1. Coat plate with 100 µL of capture antibody diluted to 10 µg/mL in Borate Buffered Saline (BBS) pH 8.4. Allow binding to occur overnight at room temperature. We recommend Corning® Costar™ 96-well clear flat bottom PVC general assay plates #2595.
2. Add blocking reagents to block plates by adding 200 µL of 1% BSA, 0.1% Tween-20 in BBS to each well. Allow blocking to occur overnight at room temperature.
3. Wash plates three times with BBS supplemented with 0.1% Tween-20.
4. Add 100 µL of collagen analyte in BBS supplemented with 0.1% Tween-20 and 0.05% SDS at 0.5 µg/mL. Allow binding to occur overnight at room temperature.
5. Wash plates three times with BBS supplemented with 0.1% Tween-20.
6. Add 100 µL of BBS to all wells except controls in preparation for serial dilution of detection antibody.
7. Add 100 µL of detection antibody diluted to 1:2,500 in BBS from a 1 mg/mL stock to the first well. Mix resulting in a 1:5,000 dilution. Perform serial two-fold dilutions across the plate. Allow binding to occur overnight at room temperature.
8. Wash plates three times with BBS supplemented with 0.1% Tween-20.
9. Add 100 µL of detection reagent Streptavidin-HRP diluted from a 1 mg/mL stock to 1:5,000 in BBS.
10. Wash plates three times with BBS supplemented with 0.1% Tween-20.
11. Add 100 µL TMBE substrate. Allow color to develop. Scan the plate to determine absorbance values.

III. Notes

Any deviation from this protocol may require additional optimization or result in loss of antibody specificity.

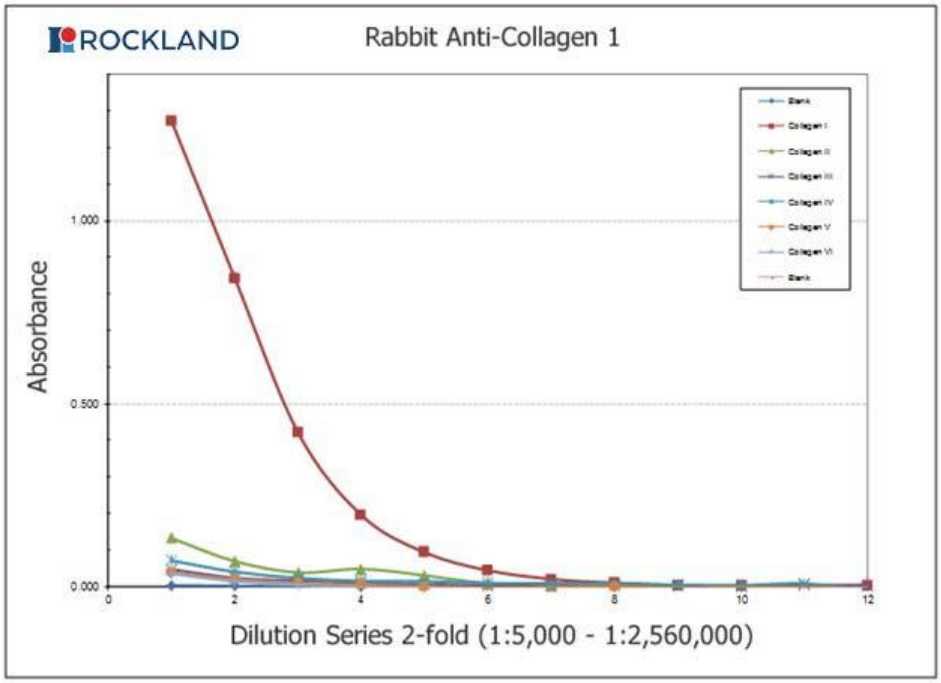

Figure: Capture ELISA using Rockland collagen antibodies designed to recognize non-denatured three-dimensional epitopes present within native collagen structure. Anti-Collagen Type I was used to capture Human Collagen Type I and other human collagens. Detection occurred using anti-Collagen Type I Biotin Conjugate followed by reaction with streptavidin HRP and TMB substrate.