ELISA Grade Type I and Type II Collagen

Catalog # 1001-1006, 2011-2017

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INTRODUCTION

Chondrex, Inc. provides highly purified, polymeric-free ELISA grade collagen. Various species and types of collagen can be used as an antigen in ELISA for studying antibody specificity and cross-reactivity (Table 1). Because collagen is a rigid fibrillar protein with unique physical and chemical properties and differs from other globular proteins, special attention for handling this protein is required. For example, immunoglobulins in human and animal sera bind to polymeric and fibrillar collagen non-specifically and create significantly high false-positive reactions in ELISA. Similarly, a secondary antibody in ELISA also binds to fibrillar collagen non-specifically and creates high background values. Furthermore, these forms of collagen significantly affect assay sensitivity due to the formation of excess collagen layers on the surfaces of ELISA plate.

ELISA grade collagen is supplied as a 100X concentrated solution (0.5 mg/ml). Each vial contains enough collagen to coat ten, 96-well plates. A 10X Collagen Dilution Buffer (Cat # 9003) is also included per order of ELISA grade collagen to prevent fibril formation.

PROCEDURE FOR COATING ELISA PLATES

1. Dilute 1 ml of 10X Collagen Dilution Buffer with 9 ml of cold (refrigerated) distilled water.

2. Add 100 µl of ELISA grade type I or type II collagen solution to the 10 ml of 1X collagen dilution buffer prepared in step 1.

3. Add 100 µl of the diluted collagen solution to each well of a 96-well ELISA plate and incubate at 4°C overnight. Cover the ELISA plate with a plate sealer or wrap to prevent evaporation.

NOTE: The binding efficiency of collagen on ELISA plates depends upon the type of plastic and pre-treatment of the well surface. Therefore, Chondrex, Inc. recommends using a high affinity binding plate such as Immulon 2 HB. Mouse monoclonal anti-type II collagen antibody (Cat # 7005), which cross-reacts to all species of type II collagen listed in Table 1, can be used as a positive control or to study the binding efficiency of individual species of type II collagen to ELISA plates. Chondrex, Inc. also provides a variety of anti-Type I and Type II collagen antibodies. Please visit www.chondrex.com for more information.

Table 1 - ELISA grade collagen provided as 1 ml x 0.5 mg/ml, 0.05M acetic acid solution

Species	Type I Collagen Catalog #	Type II Collagen Catalog #
Chick	1001	2011
Bovine	1002	2012
Porcine	1003	2013
Rat	1004	2014
Human	1005	2015
Mouse	1006	2016
Monkey	-----	2017

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TIPS FOR A SUCCESSFUL ANTI-COLLAGEN ANTIBODY INDIRECT ELISA

To accurately assay collagen antibodies in human and animal serum samples, it is important to determine intense false positive background (BG) noise reactions caused by IgG in the samples themselves in antigen uncoated wells (1). In addition, it is also important to prepare aliquoted reagents with consistent quality, which can be repeatedly used across experiments to avoid unnecessary variations between individual assays. The following are tips for successfully assaying anti-collagen antibodies by indirect ELISA.

Choosing an appropriate assay buffer: To accurately assay antibodies, it is most important to eliminate false positive BG noise reactions caused by IgG already present in the samples. For human serum samples, 0.1M Tris-buffered 100% heterologous serum, pH 7.5 can block this BG noise reaction. For mouse and rat serum samples, 2-5% heterologous serum in 0.1M PBS or TBS, pH 7.5 will serve the same purpose. In addition, heterologous serum of the same species as the secondary antibody host will reduce non-specific reactions caused by the secondary antibodies.

Critical steps in running indirect ELISAs: The following are critical steps to run indirect ELISAs with high reproducibility and accuracy using serological samples. A typical assay volume in ELISA is 100 µl/well.

1. **Coating plates with collagen**: Please refer to the plate coating section in this product sheet.

2. **Wash buffer for washing plates**: Prepare a 20X PBS-1% Tween 20 stock solution and store at room temperature. Prepare fresh 1X wash buffer by diluting with distilled water just before use.

   NOTE: Bacteria will not grow in a 20X buffer with high salt concentration.

3. **Block plates**: Using the same assay buffer chosen in Step 1, add 100 - 200 µl of the buffer into all wells and incubate at room temperature for 1 hour.

4. **Samples**: Samples should contain antibodies, such as monoclonal antibodies, polyclonal antibodies, or antibody-positive serum. Dilute the samples 1:100 with the same buffer chosen in step 1 and store at -20°C until use. If anti-collagen antibody levels are unknown, several dilutions must be tested.

5. **Plate Mapping**: ELISA plates should be mapped to determine (1) Blank (BL) values, (2) Background (BG) noise reaction values of individual samples and (3) antigen-antibody reaction values as shown in Figure 1a. If the sample dilution is less than 1/100, it is necessary to determine the BG noise reaction of individual samples in antigen non-coated wells as shown in Figure 1b.

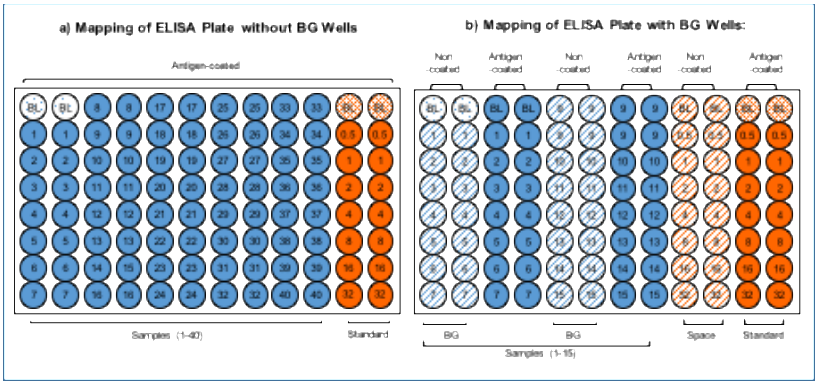

Figure 1 - Mapping of an ELISA Plate Depending on Sample Dilution**.** Blue: test sample added to antigen-coated well, Orange: Standard added to antigen-coated wells, Blue Slash: test samples added to antigen non-coated wells.

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6. **Secondary Antibody conjugated with enzymes**: Prepare a stock secondary antibody solution with 50% ethylene glycol containing 1% BSA (EG-BSA). Optimize secondary antibody concentration to obtain sufficient assay sensitivity for studies while keeping the blank OD value low. For example if the final dilution used for assay is 1/1,000 or 1/2,000 make a 1/5 or 1/10 solution, respectively (stock working solution) from the stock solution with the EG-BSA and store the stock solution at -20°C. Dilute the stock working solution from 1/5 to 1/1000 with the buffer chosen in Step 1 just before use**.**

   NOTE: Do not use 50% glycerol to prepare stock secondary antibodies because contaminants in glycerol may gradually inactivate the peroxidase conjugated to secondary antibodies during storage.

7. **Chromogens and Reading plates**: Several chromogens are available depending on the enzyme conjugated to secondary antibodies. For example, horseradish peroxidase, which is the most popular enzyme, works with multiple chromogens, but the sensitivity of the chromogens will differ. The most sensitive is 3',5,5'-Tetramethylbenzidine (TMB), followed by o-phenylenediamine dihydrochloride (OPD), with the least sensitive being 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). Each chromogen has its own appropriate absorbance to measure color densities as determined by chromogen correlated antibody concentration**.** Color is developed for 15 - 30 minutes, then terminated by adding 50 µl of 2N H_2SO_4.

8. **Comparing antibody titers**:

   1) Average the duplicate OD values for the BL, standards, and test samples.

   2) Subtract the averaged BL values from the averaged OD values of all wells of test samples and standards.

   3) If the BG noise reaction of individual samples was determined, subtract the corrected BG value in non-coated wells from the corrected OD value in the corresponding antigen-coated wells of individual test samples.

9. **Calculating antibody concentration based on standards**:

   1) Average the duplicate OD values for the BL, standards, and test samples.

   2) Subtract the averaged BL values from the averaged OD values of all wells of test samples and standards.

   3) If the BG noise reaction of individual samples was determined, subtract the corrected BG value in non-coated wells from the corrected OD value in antigen-coated wells of individual test samples.

   4) Plot the OD values of standards against the units/ml. A log/log plot will linearize the data.

   5) The concentration of antibodies (units/ml) in test samples can be calculated using regression analysis.

REFERENCES

1. K. Terato, C. Do, D. Cutler, T. Waritani, H. Shionoya, Preventing Intense False Positive and Negative Reactions Attributed to the Principle of ELISA to Re-Investigate Antibody Studies in Autoimmune Diseases. *J Immunol Methods* **407**, 15-25 (2014).

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