Human/Monkey Anti-Type I and Type II Collagen IgA Assay Kit

Catalog # 1041, 1042, 1043, 1045, 2061, 2062, 2063, 2065

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Species	Type I Collagen Color Coding - Catalog #	Type II Collagen Color Coding - Catalog #
Chick	(CI) Gold - 1041	(CII) Yellow - 2061
Bovine	(BI) Dark Blue - 1042	(BII) Green - 2062
Porcine	(PI) Brown - 1043	(PII) Pink - 2063
Human	(HI) Silver - 1045	(HII) Blue - 2065
Uncoated	Clear	Clear
Standard	Red	Red

Human sera, especially from patients with autoimmune diseases, contain high levels of immunoreactive components, which yield high background levels in ELISA systems. These non-specific reactions are caused by adhesive immunoglobulins contained in human serum, which strongly adhere to plastic surfaces by hydrophilic binding, and blocking agents such as bovine serum albumin (BSA) and Tween 20, are not capable of blocking these non-specific reactions at all. However, these false positive reactions caused by serum sample itself have not been understood, and are considered as a real antibody-antigen reaction in many cases, even now. In order to obtain a real value of antigen-antibody reaction, it is critical 1) to choose proper blocking agents which block these kinds of non-specific reactions effectively, 2) to determine a unique non-specific background value of individual samples using antigen-non-coated wells and 3) to subtract the background value from the value determined in antigen-coated wells. In addition, it is apparent to determine the non-specific reactions caused by secondary antibody as well. Chondrex's ELISA system incorporates unique blocking agents that inhibit the hydrophobic binding of these serum components onto plastic surfaces and are designed to determine the background values of individual samples using antigen-non-coated wells.

IgA antibodies to type II collagen are often associated with IgG antibodies in human sera. Importantly, IgA and IgG antibodies share identical collagen types and species specificity in individual sera, suggesting that the same collagen is involved in eliciting these antibodies. Therefore, it is highly likely that heterologous collagen in diets may play a primary role in the anti-collagen antibody production, regardless of the disease. However, this does not indicate that only type II collagen is always the eliciting antigen, since type I collagen, found abundantly in diets, share amino acid sequences with type II collagen by more than 80% and the antibodies in human sera often react to both type I and type II collagen. To determine the diversity of anti-collagen antibodies in human sera, Chondrex provides IgA and IgG antibody assay kits with various species of type I and type II collagen-coated strips as well as uncoated wells (see table above). This ELISA kit contains enough materials to run two plates on two separate occasions (see assay procedure) and may be used for monkey sera as well as human sera.

Note: Since IgA and IgG antibodies in human sera share similar collagen types and species of specificity, it is assumed that IgA antibodies determined by this ELISA kit might be underestimated due to the competitive binding of IgG antibodies to the identical epitopes on collagen molecules. In order to determine accurate IgA antibody levels in human serum samples, it is recommended to treat serum samples with Protein G to remove IgG antibodies.

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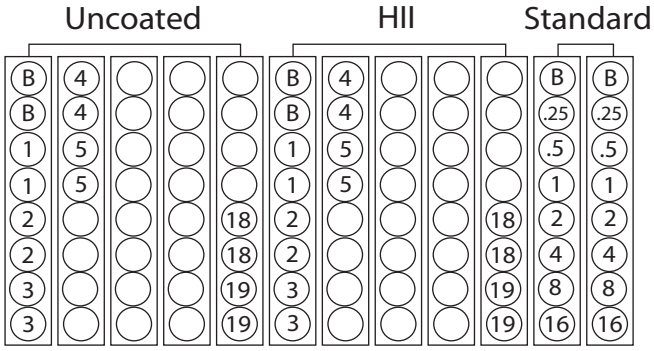
Standard ELISA Kit with One Species of Type I or Type II Collagen

Figure 1 shows a standard ELISA kit consisting of five 8-well strips which are uncoated and serve as a control for background levels of individual samples, five 8-well strips coated with one species of type I or type II collagen to determine specific antibody levels, and two 8-well strips for reference standards. "B" represents blank wells to determine non-specific reactions caused by the secondary antibody. Standards and samples are run in duplicate.

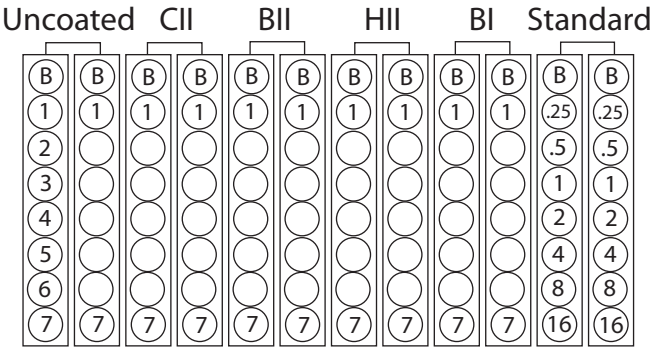
Figure 1 - Standard ELISA kit coated with a single species of type II collagen



Custom ELISA Kit with Multiple Species of Collagen

Figure 2 shows an example of a custom kit for assaying antibody levels to various species of type I or type II collagen in human serum. This custom ELISA plate consists of two uncoated 8-well strips, two each of 8-well strips coated with chick (CII), bovine (BII) and human (HII) type II collagen, as well as bovine (BI) type I collagen and two 8-well strips for reference standards. "B" represents blank wells to determine non-specific reactions caused by the secondary antibody.

Figure 2 - Custom ELISA system for assaying species specificity of collagen antibodies in human sera



KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody	1 vial	1.1 mL, 16 units/mL	-20°C
Secondary Antibody (Biotin-Conjugated Goat Anti-Human IgA)	2 vials	50 µL, lyophilized	-20°C
Solution A - Blocking Buffer	1 bottle	20 mL	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	50 mL	-20°C
Solution C1 - Secondary Antibody Dilution Buffer	1 bottle	20 mL	-20°C
Solution C2 - Streptavidin Peroxidase Dilution Buffer	1 bottle	20 mL	-20°C
Streptavidin Peroxidase	2 vials	50 µL	-20°C
OPD	2 vials	Lyophilized	-20°C
OPD Dilution Buffer	1 bottle	20 mL	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 mL	-20°C
Wash Buffer, 20X	2 bottles	50 mL	-20°C
Type I or Type II Collagen-Coated 8-Well Strips	10 each	8-well strips	-20°C
Uncoated 8-Well Strips	10 each	8-well strips	-20°C
Reference Standard Strips (two strips per run)	4 each	8-well strips	-20°C

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NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Partially used reagents may be kept at –20°C.

Note 3: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, it is necessary to warm the wash buffer by placing the bottle in warm water until crystals have dissolved completely.

Note 4: Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.

ASSAY PROCEDURE

1. **Dilute Wash Buffer**: Dilute 50 mL of 20X wash buffer in 950 mL of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

2. **Add Blocking Buffer**: Add 100 μL of Blocking Buffer (Solution A) to all wells. Incubate for 1 hour at room temperature.

3. **Prepare Standard Dilutions**: Undiluted standard is 16 units/mL. Prepare serial dilutions of the standard by mixing 250 μL of 16 units/mL standard with 250 μL of Sample/Standard Dilution Buffer (Solution B) - 8 units/mL . Then repeat this procedure to make five more serial dilutions of standard - 4, 2, 1, 0.5 and 0.25 units/mL solutions. The 16 units/mL standard may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.

4. **Prepare Sample Dilutions**: Dilute samples 1:100 or more with Solution B. For example, dilute 20 μL of sample with 1.98 mL of Solution B (1:100). Keep this as a stock solution for future assays. If necessary, dilute the samples further with Solution B, 1:200-1:1000.

   Note: Human serum samples have a high lipid content in general. In order to avoid non-specific reactions caused by lipids, centrifuge samples at 10,000 rpm for 5 minutes using a tabletop centrifuge. Take a desired volume of serum sample carefully by pipette and then wipe the pipette surface with paper.

5. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

6. **Add Standards and Samples**: Add 100 μL of standards, Solution B (blank), and samples to collagen coated and uncoated wells in duplicate. Incubate at 4°C overnight.

7. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

8. **Add Secondary Antibody**: Dissolve one vial of secondary antibody in 10 mL Secondary Antibody Dilution Buffer (Solution C1). Add 100 μL of secondary antibody solution to each well and incubate at room temperature for 2 hours.

9. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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10. **Add Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 mL of Streptavidin Peroxidase Dilution Buffer (Solution C2). Add 100 μL of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

11. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

12. **OPD**: Dissolve one vial of OPD in 10 mL of OPD Dilution Buffer just prior to use. Add 100 μL of OPD solution to each well immediately after washing the plate. Incubate for 30 minutes at room temperature.

13. **Stop**: Add 50 μL of 2N sulfuric acid (Stop Solution) to each well.

14. **Read Plate**: Read the OD values at 490 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the standards, blanks (B) and test samples in uncoated wells and collagen coated wells.

2. Subtract the blank (B) values from the averaged OD values of the standards and test samples in uncoated wells and collagen coated wells.

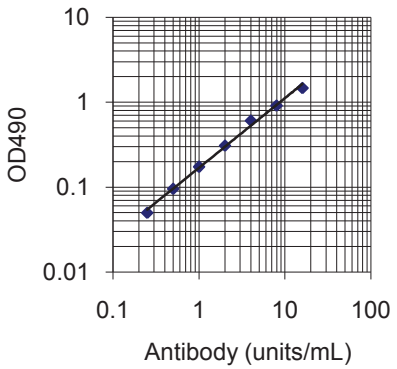
   Note: Individual antigens have unique background values. Therefore, blank wells should be used for each different antigen.

3. Subtract the OD values of samples tested in uncoated wells (background values) from their counterpart OD values in collagen coated wells from step 2 to eliminate values associated with non-specific reactions.

4. Plot the OD values of standards against the units/mL of antibody standard. Using a log/log plot will linearize the data. Figure 3 shows a representative experiment where the standard range is from 0.25 to 16 units/mL.

5. The units/mL of antibody in test samples can be calculated using regression analysis.

Figure 3 - A typical standard curve



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