Human/Monkey Anti-Type I and Type II Collagen IgA Antibody ELISA Kits with TMB

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

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify human/monkey anti-collagen antibodies
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	5.25 hours
STANDARD RANGE:	16 units/ml to 0.25 units/ml
NUMBER OF SAMPLES:	Up to 38 (duplicate) samples/standard plate (will vary for custom kits)
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	

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INTRODUCTION

Human serum, 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 in human serum which so strongly adhere to plastic surfaces by hydrophilic binding that blocking agents such as bovine serum albumin (BSA) and Tween 20 are not capable of blocking these non-specific reactions at all. False positive reactions caused by the serum samples themselves are usually overlooked and are considered real antibody-antigen reactions in many cases, even now. In order to obtain the real values of antigen-antibody reactions, it is critical 1) to choose proper blocking agents which block these kinds of non-specific reactions effectively, 2) to determine the unique non-specific background value of each individual sample using antigen-non-coated wells, and 3) to subtract that background value from the corresponding value determined in antigen-coated wells. In addition, it is important to determine the non-specific reactions caused by the secondary antibody as well. Chondrex, Inc.'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 serum. Importantly, IgA and IgG antibodies share identical collagen types and species specificity in individual serum, 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 mean that type II collagen is always the eliciting antigen as type I collagen, commonly found in diets, share's over 80% of its amino acid sequence with that of type II collagen, and the antibodies in human serum often react to both type I and type II collagen. To determine the diversity of anti-collagen antibodies in human serum, Chondrex, Inc. provides IgA and IgG antibody ELISA kits with various species of type I and type II collagen-coated strips as well as uncoated wells. This ELISA kit contains enough materials to run two plates on two separate occasions and may be used for monkey serum as well as human serum.

NOTE: Because 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 accurately determine IgA antibody levels in human serum samples, Chondrex, Inc. recommends treating serum samples with Protein G to remove IgG antibodies.

PLATE COATING AND SETUP

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

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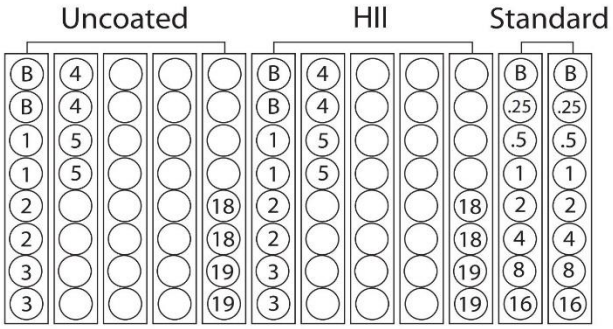
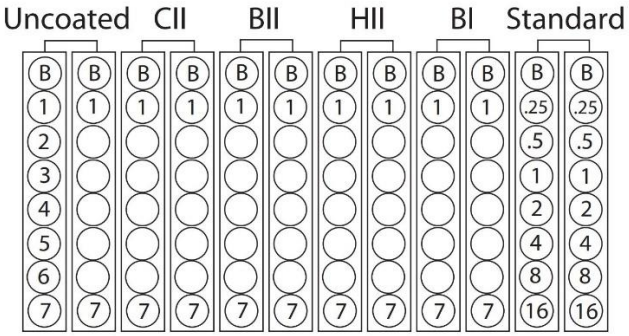
Standard ELISA Kit with One Species of Type I or Type II Collagen

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.



Custom ELISA Kit with Multiple Species of Collagen

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.



KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard IgA Antibody (7011)	1 vial	1.1 ml, 16 units/ml	-20°C
Secondary Antibody (Biotin-Conjugated Goat Anti-Human IgA) (7038)	2 vials	Lyophilized	-20°C
Solution A - Blocking Buffer (9027)	1 bottle	20 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (9038)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (9039)	1 bottle	20 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 ml	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	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: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

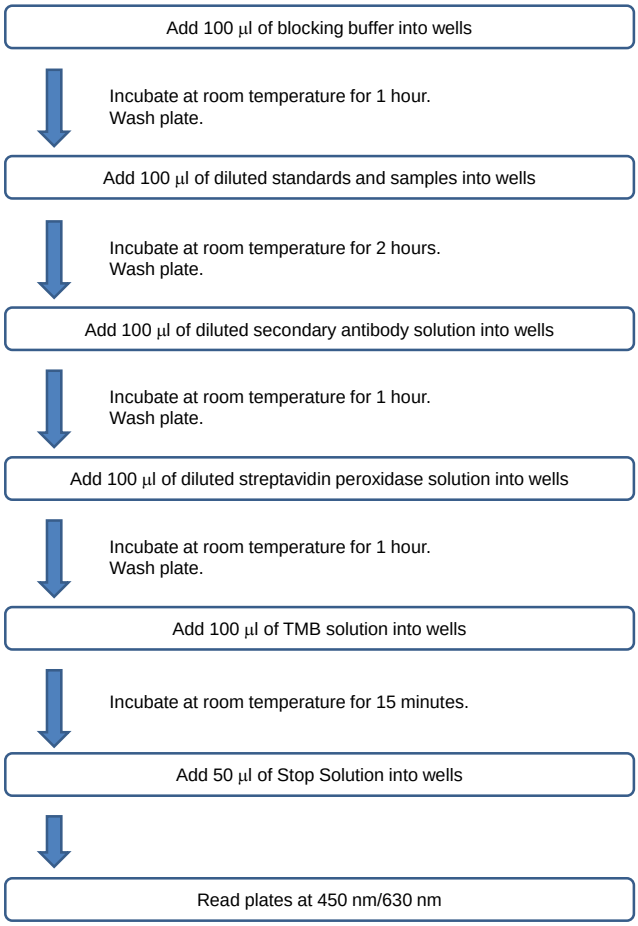
NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY OUTLINE



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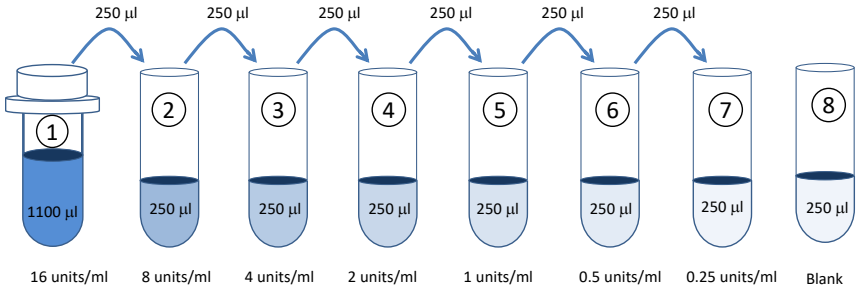
ASSAY PROCEDURE

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

2. **Prepare Standard Dilutions**: The undiluted standard stock solution is 16 units/ml. Prepare serial dilutions of the standard by mixing 250 µl of the 16 units/ml standard with 250 µl of 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. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **Prepare Sample Dilutions**: If necessary, centrifuge serum samples at 10,000 rpm at room temperature for 3 minutes to remove insoluble materials and lipids. Dilute samples 1:100 or more with Solution B. For example, dilute 10 µl of sample with 0.99 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.

4. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Standards and Samples:** Add 100 µl of standards, Solution B (blank) and samples to wells in duplicate. Incubate at room temperature for 2 hours.

6. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Secondary Antibody:** Dissolve one vial of secondary antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial in 50 µl of Solution C and dilute accordingly. Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Secondary Antibody (µl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

8. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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

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Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

10. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **TMB:** Dilute one vial of TMB in 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to each well immediately after washing the plate. Incubate for 15 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

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

13. **Read Plate:** Read the OD values at 450 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 1 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.

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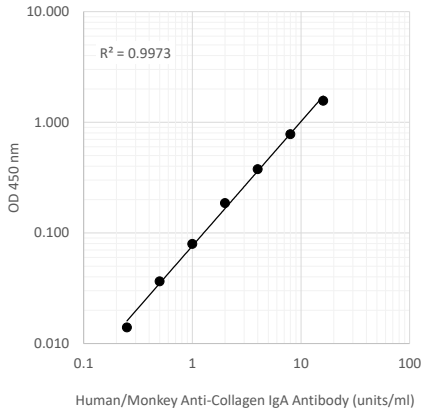
Figure 1 - A Typical Standard Curve for the Human/Monkey Anti-Collagen IgA ELISA Kit.



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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