Chondrex, Inc.

Rat Anti-Type I and Type II Collagen IgG Assay Kit

Catalog # 1021-1025, 2041-2045

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Heterologous type II collagen is widely used as an immunogen for inducing arthritis, so called collagen-induced arthritis (CIA). In CIA-susceptible strains of rats, the serum antibody levels against the type II collagen used for immunization are very high, and cross-react to various species of type II collagen including homologous type II collagen, due to the highly conserved amino acid sequences of type II collagen.

Species	Type I Collagen Color Coding - Catalog #	Type II Collagen Color Coding - Catalog #
Chick	(CI) Gold - 1021	(CII) Yellow - 2041
Bovine	(BI) Dark Blue - 1022	(BII) Green - 2042
Porcine	(PI) Brown - 1023	(PII) Pink - 2043
Rat	(RI) Lime Green - 1024	(RII) Purple - 2044
Human	(HI) Silver - 1025	(HII) Blue - 2045
Standard	Red	Red

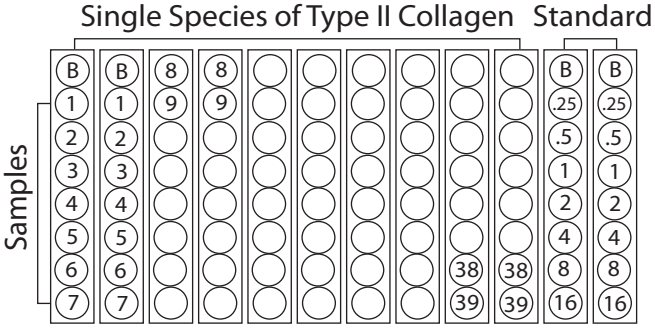
Importantly, although type I collagen shares the same amino acid sequences with type II collagen by more than 80%, it is not capable of inducing autoimmune-mediated diseases. This indicates that epitope specificity of antibodies and T-cells are important for establishing autoimmunity and subsequent development of autoimmune diseases. Therefore, type I collagen might be useful as a control for studying the pathogenesis of autoimmune mediated arthritis, and B and T-cell epitope specificity.

This kit is designed to assay type I and type II collagen antibodies in rat sera. Chondrex's ELISA systems incorporate unique blocking agents to reduce non-specific reactions. These agents reduce the background levels by inhibiting the hydrophobic binding of immunoreactive serum components in sample specimens onto plastic surfaces. Various species of type I and type II collagen-coated strips are available as shown in the table above. This ELISA kit contains enough materials to run two plates on two separate occasions (see assay procedure).

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

Figure 1 shows a standard ELISA kit consisting of ten 8-well strips coated with one species of type II collagen and two 8-well strips for reference standards. "B" represents blank wells to determine background values caused by the secondary antibody. Standards and samples (1-39) are run in duplicate.

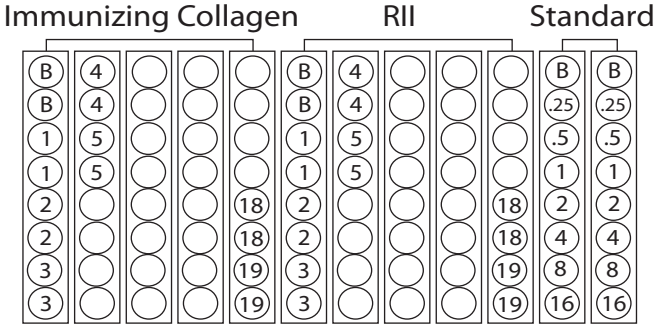
Figure 1 - Standard ELISA kit coated with a single species of type II collagen



Custom ELISA Kit with Multiple Species of Collagen

Figure 2 shows an example of a custom kit for assaying antibody levels to the immunizing type II collagen and their cross-reactivity to homologous rat type II collagen (RII). "B" represents blank wells to determine background values caused by the secondary antibody. Standards and samples (1-19) are run in duplicate.

Figure 2 - Custom ELISA kit for assaying antibodies to various species of collagen



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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody	1 vial	20 units, lyophilized	-20°C
Secondary Antibody (Peroxidase-Conjugated Goat Anti-Rat IgG)	2 vials	50 µl, lyophilized	-20°C
Solution A - Blocking Buffer	1 bottle	12 ml	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer	1 bottle	20 ml	-20°C
OPD	2 vials	Lyophilized	-20°C
Chromagen Dilution Buffer	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 ml	-20°C
Wash Buffer, 20X	1 bottle	50 ml	-20°C
Type I or Type II Collagen-Coated 8-Well Strips	10 each	8-well strips	-20°C
Reference Standard Strips (two strips per run)	4 each	8-well strips	-20°C

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**: Dissolve one vial of standard (20 units/vial) in 1.25 ml of Sample/Standard Dilution Buffer (Solution B) to make 16 units/ml solution. Prepare serial dilutions of the standard by mixing 250 µl of 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. We recommend making fresh serial dilutions for each assay.

4. **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 10 µl of sample with 0.99 ml of Solution B (1:100). Keep this as a stock solution for future assays. Then, further dilute the stock solution with Solution B; for example, 1:20,000-1:80,000 for serum samples from arthritic rats.

   Note: The serum antibody levels differ significantly among individual strains of rats and timing of serum collection after immunization. It is recommended to optimize the dilution using selected samples before running a large number of samples.

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 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 C). 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.*

10. **OPD**: Dissolve one vial of OPD in 10 ml of Chromagen 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.

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

12. **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.

2. Subtract the blank (B) values from the averaged OD values of the standards and test samples.

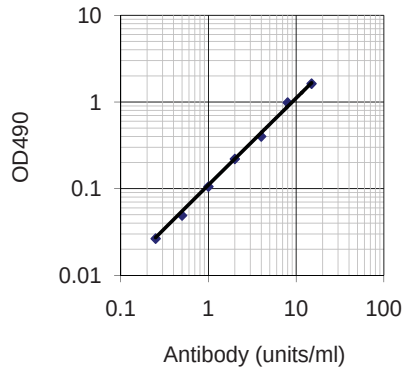
   Note: Individual antigens have unique background values. Therefore, blank wells should be used for each different antigen.

3. 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.

4. The units/ml of antibody in test samples can be calculated using regression analysis.

   Note: 82 units is approximately 1 µg IgG antibody/ml.

Figure 3 - A typical standard curve



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