

For Research Use Only

## Mouse IgG Anti-T type I and T type II Collagen Assay Kit

Catalog # 1011, 1012, 1013, 1015, 1016, 2031, 2032, 2033, 2035, 2036

### INTRODUCTION

This kit is designed to assay type I and type II collagen antibodies in mouse sera. The Arthrogen-CIA® 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 on the right. This ELISA kit contains enough material to run two partial plates on two separate occasions (see assay procedure).

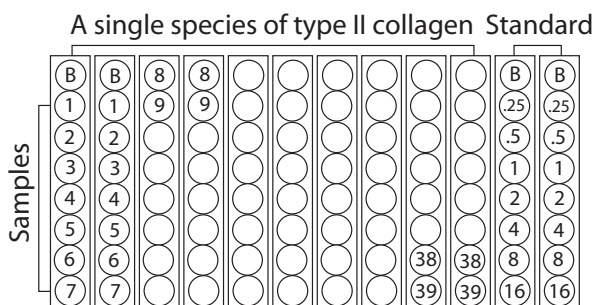
Heterologous type II collagen is widely used as an immunogen for the collagen-induced arthritis (CIA) model. In CIA-susceptible mice, the serum antibody levels to the type II collagen used for immunization are very high. Furthermore, these antibodies cross-react to various species of type II collagen including autologous type II collagen, due to the conserved amino acid sequences of type II collagen. 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. However, type I collagen might be useful as a control to study the pathogenesis of autoimmune-mediated arthritis and the antibody epitopes on collagen.

Species	Type I Collagen Color Coding	Type II Collagen Color Coding
Chick	(CI) Gold	(CII) Yellow
Bovine	(BI) Blue	(BII) Green
Porcine	(PI) Brown	(PII) Pink
Human	(HI) Silver	(HII) White
Mouse	(MI) Black	(MII) Orange
Rat	(RI) Lime Green	(RII) Purple
Uncoated	Clear	Clear
Standard	Red	Red

### 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 (numbers 1 - 39) are run in duplicate.

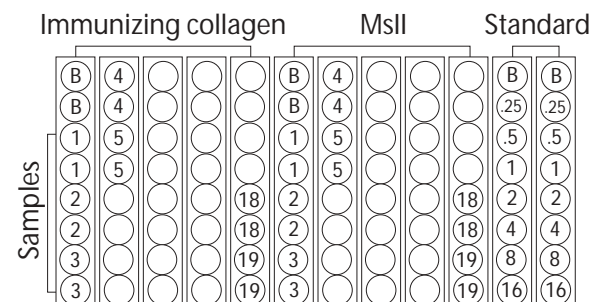
Figure 1 - Standard ELISA kit coated with a single species of type II collagen



### 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 mouse type II collagen (MII). "B" represents blank wells to determine background values caused by the secondary antibody. Standards and samples (numbers 1 - 19) are run in duplicate.

Figure 2 - Custom ELISA system for assaying antibodies to various species of type II collagen



## KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody	1 vial	1.1 ml, 16 units/ml	-20°C
Secondary Antibody (peroxidase-conjugated goat anti-mouse IgG)	2 vials	50 ul lyophilized	-20°C
Solution A - Blocking Buffer	1 bottle	12 ml	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	60 ml	-20°C
Solution C - Secondary Antibody 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
OPD Chromagen and Urea H <sub>2</sub> O <sub>2</sub> Buffer Tablets	1 vial	2 tablets each	-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

## ASSAY PROCEDURE

It is recommended that standards and samples be run in duplicate. Unused strips for standards and test samples should be kept in the dark at -20°C.

*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 buffer bottle in warm water until all crystals have dissolved completely.*

- Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 1 liter of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times. **Do not allow the plate to dry out**, proceed quickly to step 2.
- Add Blocking Buffer:** Add 100 µl of Blocking Buffer (Solution A) to all wells. Incubate for 1 hour at room temperature.
- Prepare Standard Dilutions:** Prepare serial dilutions of the standard using the Sample/Standard Dilution Buffer (Solution B). Undiluted standard is 16 units/ml. Mix 250 µl of the standard with an equal volume of Solution B to make 8 units/ml solution. Then repeat this procedure to make 4, 2, 1, 0.5, and 0.25 units/ml solutions.
- Prepare Sample Dilutions:** Centrifuge serum samples at 10,000 rpm at room temperature for 3 minutes to remove insoluble materials and lipids. Dilute samples 1:1,000 or more with Solution B. For example, dilute 10 µl of sample with 990 µl of Solution B (1:100). Keep this as a stock solution. Higher dilutions as determined by the user may be made from this stock solution. If sample is diluted less than 1:200 due to low antibody levels, please contact Chondrex Customer Service.
- Wash:** Wash plate with 1X wash buffer at least 3 times immediately prior to adding blanks, samples, and standards. **Do not allow plate to dry out.**
- Add Blanks:** Add 100 µl of Solution B to the wells labeled "B" as shown in Figures 1 and 2. These are the background control wells.

7. **Add Samples:** Add 100  $\mu$ l of the diluted test samples to the wells (labeled 1 - 39) as shown in Figures 1 and 2. Samples should be tested in duplicate.
8. **Add Standards:** Add 100  $\mu$ l of the standard solutions (0.25 - 16 units/ml) to the wells of the standard strip (red color coded) as shown in Figures 1 and 2. Standards should also be tested in duplicate.
9. **Incubate** overnight at 4°C.
10. **Prepare Secondary Antibody:** Dissolve one vial of Secondary Antibody in 10 ml of Secondary Antibody Dilution Buffer (Solution C).
11. **Wash:** Wash the plate with 1X wash buffer at least 6 times just prior to adding the secondary antibody. **Do not allow plate to dry out.**
12. **Add Secondary Antibody:** Add 100  $\mu$ l of secondary antibody solution into all wells and incubate for 2 hours at room temperature.
13. **Prepare OPD-Urea H<sub>2</sub>O<sub>2</sub>:** Lightly crush one tablet of urea H<sub>2</sub>O<sub>2</sub> buffer tablet in its packet. Dissolve all contents of the urea H<sub>2</sub>O<sub>2</sub> tablet and one OPD tablet together into 20 ml of room temperature glass distilled water immediately prior to use. **This solution is not stable and should be used within 15 minutes.**
14. **Wash:** Wash the plate with 1X wash buffer at least 6 times. **Do not allow plate to dry out.**
15. **Add OPD-Urea H<sub>2</sub>O<sub>2</sub>:** Add 100  $\mu$ l of OPD-urea H<sub>2</sub>O<sub>2</sub> solution to each well immediately after washing the plate and incubate for 30 minutes at room temperature.
16. **Stop:** Stop the reaction with 50  $\mu$ l of 2N sulfuric acid (Stop Solution).
17. **Read Plate:** Read the OD values at 490 nm. If the OD values are greater than 2.0, re-assay the sample at a higher dilution. For best results, plates should be read as soon as possible.

Note: A 650 nm filter can be used as a reference.

## CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the background, standards, and samples.
2. Subtract the background (B) values from the averaged OD values in step 1.
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: 100 units is approximately 0.1  $\mu$ g IgG antibody/ml.

Figure 3 - A typical standard curve

