Rat Anti-Type II Collagen ELISA Kit
Catalog Number: ASB-6000-EX

ELISA Assay Kit to measure rat IgG antibodies to type II collagen.

INTRODUCTION

Collagen-induced arthritis (CIA) is a model of chronic inflammatory arthritis which closely resembles human rheumatoid arthritis (RA). Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening therapeutics. This model also has the primary advantage that the mechanisms of pathogenesis are known; susceptible mice, rats or monkeys (Trentham, 1982; Cathcart *et al*., 1986; Yoo *et al*., 1988) develop arthritis after immunization with heterologous, native type II collagen. For mice, collagen is emulsified with complete Freund's adjuvant which is injected s.c. at the base of the tail. The onset of arthritis occurs within 4 to 5 weeks. For rats, collagen is emulsified in incomplete Freund's adjuvant (since rats are susceptible to adjuvant arthritis) and the onset of arthritis occurs within 10 days to 3 weeks, depending upon the strain of rat used. Clinical signs of arthritis include red and swollen paws. Histopathological features include infiltration of inflammatory cells, pannus formation and cartilage and bone destruction.

The immunopathogensis of CIA is initiated when crossreactive antibodies, mounted against the heterologous type II collagen, bind to the host's cartilage, activate complement and subsequently the inflammation cascade consisting of pro-inflammatory cytokines, chemokines and matrix matrix-degrading enzymes. Since anti-collagen antibodies are the effectors of inflammation, their presence in serum directly correlates with the onset of arthritis and provides a useful clinical marker. The Rheumera™ ELISA system allows the measurement of anti-type II collagen antibodies in serum from mice. The kit can be ordered with various species of type II collagen (see chart below).

PRINCIPLE OF THE ASSAY

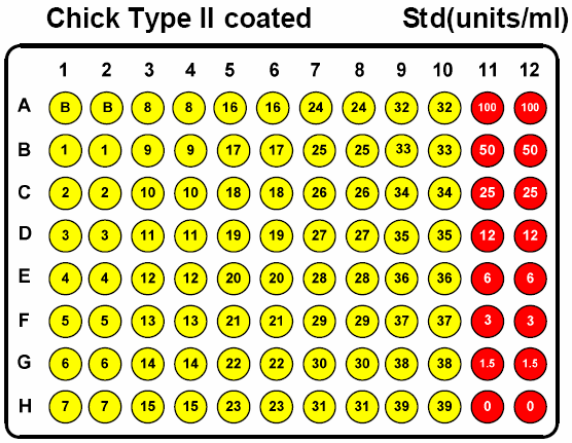
This assay measures antibodies to native type II collagen from serum using enzyme-linked immunosorbent assay (ELISA). The kit will measure 39 samples in duplicate. Removable strips and aliquoted reagents allow for samples to be tested on 2 partial plates on 2 separate occasions. Samples are incubated in wells that are coated with type II collagen which have been first pre-treated with blocking buffer. Standards are also incubated in wells that are coated with type II collagen. The wells are washed to remove unbound antibodies, and then incubated with a secondary antibody which is anti-rat IgG conjugated to peroxidase. The wells are washed to remove unbound secondary antibodies and then incubated with a chromogen substrate solution, TMB. A blue color develops which then turns yellow when the stop solution, dilute sulfuric acid, is added. The color intensity is proportion to the amount of anticollagen antibody bound to type II collagen. The sample values, measured as Units/ml, are determined by the standard curve.

Kits can be ordered with single or multiple species of type II collagen as depicted in the following chart:

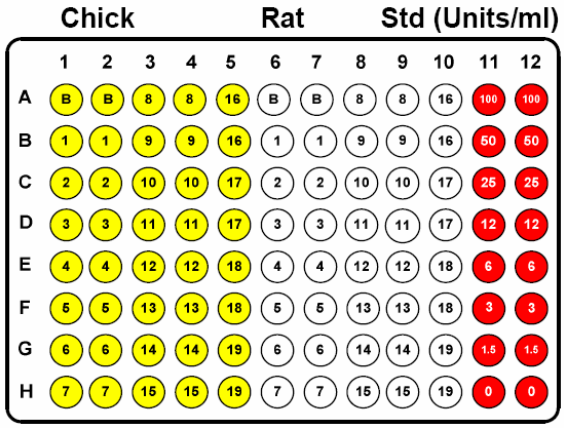
Species of Type II Collagen	Color Code
Standard	Red
Chicken	Yellow
Bovine	Green
Porcine	Purple
Human	Blue
Rat	White
Mouse	Black
uncoated	Clear (no color)

EXAMPLES OF DIFFERENT ELISA PLATE CONFIGURATIONS.

1. A <u>standard</u> ELISA plate where samples and standards are run in duplicate. Ten, 8-well strips are coatedwith the species of type II collagen (in this example, chicken) used for immunization. Two, 8well strips are used for the standards (0 to 100 units/ml). "B" represents blank wells to determine secondary antibody background levels.



2. A <u>custom</u> ELISA plate where samples and standards are run in duplicate. Five, 8-well strips are coated with the species of type II collagen (in this example, chicken) used for immunization. Five, 8well strips are coated with rat type II collagen to measure autoreactive antibodies. Two, 8-well strips are used for the standards (0 to 100 units/ml). "B" represents blank wells to determine secondary antibody background levels.



REAGENTS INCLUDED

A. Type II collagen-coated strips (10). See above chart for color coding.
B. Standard coated strips (4).
C. Blocking/Diluent Buffer 1X (60ml). Solution
D. Wash Buffer 10X (50ml). Solution
E. Secondary Ab (2 vials). Lyophylized. Anti-rat IgG, conjugated to peroxidase. Use 1 vial for each half plate (both vials for a full plate).
F. **Standard (1 vial).** Lyophylized.
G. TMB (1X) - 10ml. Chromogen substrate for peroxidase detection.
J. Stop Solution (1X) – 5ml. Dilute sulfuric acid to stop the color reaction.

REQUIRED EQUIPMENT/LABWARE/REAGENTS

Microplate reader with 450 nm and 540 nm or 650 nm reference filters.
Single and multi-channel pipettes and tips.
Deionized or distilled water.
Wash bottle with 8 or 12 spigot manifold dispenser or automated microplate washer.
Graduated cylinder.

SAMPLE/ REAGENT PREPARATION

Bring all reagents to room temperature before use.

Sample Preparation. The dilution range for samples will vary greatly depending upon the immunization antigen, dose, and frequency. A recommended range is 1:1000 to 1:100,000 in **Blocking/Diluent Buffer (C)**.

Solution D. Wash Buffer (10X) - 50ml. Dilute the contents in 450ml dH_2O to make 1X.

Solution E. Anti-Rat IgG Ab. Lyophylized. For a half plate, dilute the contents of one vial in 5.0 ml **Blocking/Diluent Buffer (C)**. For a full plate, use both vials. **IMPORTANT NOTE** * Make sure to rinse the vial several times with **Blocking/Diluent Buffer (C)** to ensure that all lyophilized material is in solution.

Solution F. Standard. Add 1.0ml of **Blocking/Diluent Buffer (C)** and vortex well. This solution is 100 Units/ml. Prepare serial dilutions of the standard by mixing 250 ml of the 100 Units/ml standard with 250 ml of **Blocking/Diluent Buffer (C)** in a separate tube to make 50 Units/ml solution. Repeat this procedure to make 25, 12.5, 6.25, 3.125 and 1.6 Units/ml. 0 Units/ml is **Blocking/Diluent Buffer (C)** only. The unused 100 Units/ml standard may be stored at 4°C for up to one month for use in a second assay.

PRECAUTIONS

The following agents are hazardous, wear appropriate eye, hand, face, and clothing protection:

Solution H (dilute sulfuric acid).
Solution G (TMB).

ASSAY PROCEDURE

It is recommended that test samples and positive controls be run in duplicate.

1. Prepare Samples and Reagents as described above.

2. Blocking. Add 100 µl of **Blocking/Diluent Buffer (C)** to all wells. Incubate for 30 minutes at room temperature. Empty the plate by inverting it and blot on a paper towel.

3. Sample and Standards (see Examples 1 and 2 above). Add 100 µl of diluted samples to type II collagen-coated wells. Add 100 µl of diluted standards to Standard-coated wells (Red). Incubate for 2 hours at room temperature or overnight at 4°C to achieve better results (i.e. higher OD values).

4. Wash 3 times with **1 X Wash Buffer (D)** using a wash bottle with manifold or automated plate washer. Blot the inverted plate on a paper towel to remove excess liquid.

5. Secondary Ab (E). Add 100 µl of diluted anti-rat IgG conjugated to peroxidase to all wells and incubate for 1-2 hours at room temperature.

6. Wash 3 times with **1 X Wash Buffer (D)** using a wash bottle with manifold or automated plate washer. Blot the inverted plate on a paper towel to remove excess liquid.

7. Chromogen. Add 100 µl of **TMB (G)** to all wells and incubate 30 minutes at room temperature.

8. Stop reaction. Add 50 ・l of **Stop Solution (H)** to each well.

9. Read OD value at 450 nm (A 540 nm or 650 nm filter can be used as a reference).

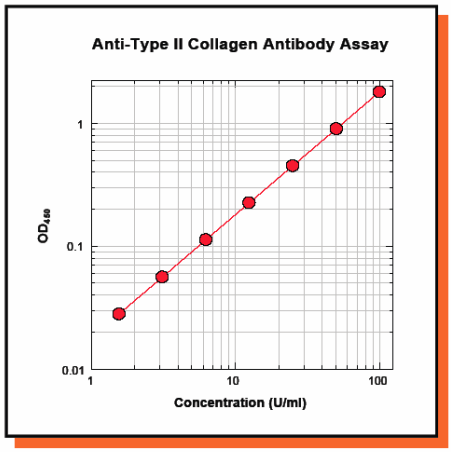
CALCULATION OF RESULTS

1. Average the duplicate OD values for the blanks (B), standards and test samples.

2. Subtract the blank (B) values from the averaged OD values in step 1. The blank (B) wells for each different species of collagen should be used, if more than one species is used.

3. Plot the OD values of standards against the Units/ml of antibody standard. Using a log/log plot will make the data linear. The figure to the right shows a representative experiment where the standard range is from 0 to 100 Units/ml.

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



A typical standard curve

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