Rat Anti-Type II Collagen IgG Subtype Antibody ELISA Kits

Catalog # 20421T, 20422T, 20431T, 20432T, 20441T, 20442T

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify rat anti-collagen antibodies
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.25 hours
STANDARD RANGE:	16 units/ml to 0.25 units/ml
NUMBER OF SAMPLES:	Up to 39 (duplicate) samples/standard plate (will vary for custom kits)
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:1000 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	

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INTRODUCTION

Autoantibodies to type II collagen play a primary role in the collagen induced arthritis (CIA) model. However, autoantibodies are not always capable of inducing arthritis due to the inability to activate a complement, the first critical step in the activation of inflammatory cascades. Therefore, instead of IgG1, the production of IgG2a and IgG2b subtype autoantibodies which are capable of activating the complement cascade is essential in the development of arthritis. In fact, the immunization of type II collagen emulsified with incomplete Freund's adjuvant develops severe arthritis in rats by primarily eliciting IgG2a and IgG2b antibodies against type II collagen. However, pretreatment with immunization of type II collagen mixed with aluminum adjuvant reduces severity of arthritis by inducing an anti-collagen antibody subclass shift from IgG2a and IgG2b to IgG1 (1). These results demonstrate the importance of determining the IgG subtype of the antibodies against the heterologous collagen used for immunization as well as the rat type II collagen for the evaluation of the immune response in the rat CIA model (2). In addition, this is especially important for the evaluation of immune modulating adjuvants which can be used for a treatment of autoimmune diseases such as RA (3).

Chondrex provides Rat Anti-Collagen IgG Subtype ELISA Kits for further analysis of antibodies in rat serum from the rat CIA model. These kits can also be used to determine antibodies to type I collagen upon request. Please contact Chondrex, Inc. customer service at support@chondrex.com for more information on custom type II collagen and type I collagen-coated plates.

Note: The antibody-antigen affinity may vary significantly among serum samples and the IgG subtypes may recognize different epitopes. Therefore, the total IgG concentration calculated as the sum of the IgG subtypes may not be the same as the total IgG levels determined by the Rat Anti-Collagen IgG ELISA Kits.

KIT COMPONENTS

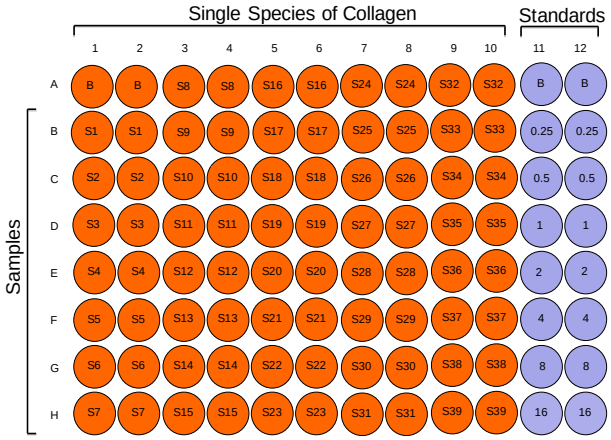
Item	Quantity	Amount	Storage
Standard Antibody IgG1 (20401) or IgG2a (20403)	1 vial	20 units, lyophilized	-20°C
Secondary Antibody IgG1 (20402) or IgG2a (20404)	2 vials	50 µl	-20°C
Solution A - Blocking Buffer (2071)	1 bottle	12 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (2072)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (2073)	1 bottle	20 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)	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

PLATE COATING AND SETUP

Type II Collagen Color Coding – Catalog #	IgG1 Catalog #	IgG2a Catalog #
(Rat) Purple	20441T	20442T
(Bovine) Green	20421T	20422T
(Porcine) Pink	20431T	20432T
(Standard) Red	-	-

Standard ELISA Kit with One Species of Type I or Type II Collagen
A standard ELISA kit for assaying an individual anti-collagen IgG subtype antibody level. Each kit contains one 96-well plate coated with one species of type II collagen for each IgG subtype antibody assay. In addition, two extra 8-well standard strips are included per subtype for two separate assays. "B" represents blank wells to determine background values caused by the secondary antibody. Standards and samples (numbers 1 - 39) are run in duplicate.

NOTE: Custom coating of plates is available upon request.



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 -20°C 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

Add 100 µl of blocking buffer into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted secondary antibody solution into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of TMB solution into wells

↓ Incubate at room temperature for 15 minutes.

Add 50 µl of Stop Solution into wells

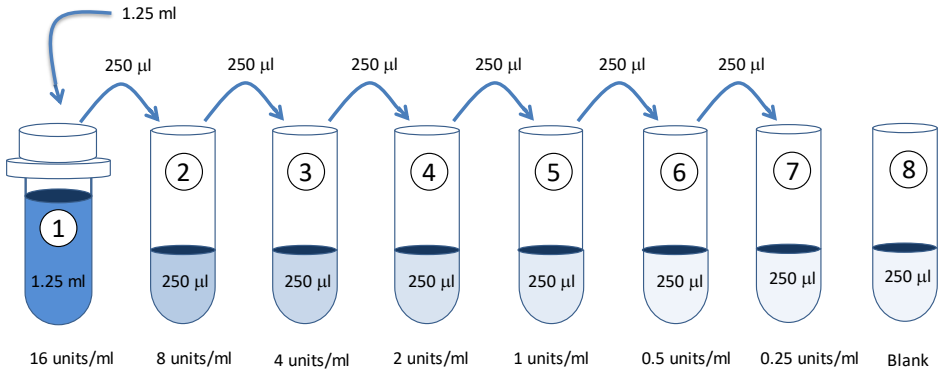
↓

Read plates at 450 nm/630 nm

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**: Dissolve one vial of standard (20 units/vial) in 1.25 ml of Sample/Standard Dilution Buffer (Solution B) to make a 16 units/ml solution. 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.



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3. **Prepare Sample Dilutions**: Centrifuge serum samples at 10,000 rpm at room temperature for 3 minutes to remove insoluble materials and lipids. Dilute samples 1:1000 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 it is necessary to assay antibodies at a low dilution (less than 1:200) due to low antibody levels, please contact Chondrex, Inc. customer service at support@chondrex.com. In the CIA model, anti-collagen antibody levels may vary from zero (naïve rodents) to 1,000,000 units/ml or higher (arthritic rodents) depending on the immune response, arthritis severity, and time of sample collection. With unknown anti-collagen antibody levels, Chondrex, Inc. recommends testing several dilutions, such as 1:1000, 1:10,000, and 1:100,000, in order to find the appropriate dilution for the samples.

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:** Dilute 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 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. **TMB:** Use new tubes when preparing TMB. Dilute one vial (200 µl) 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

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

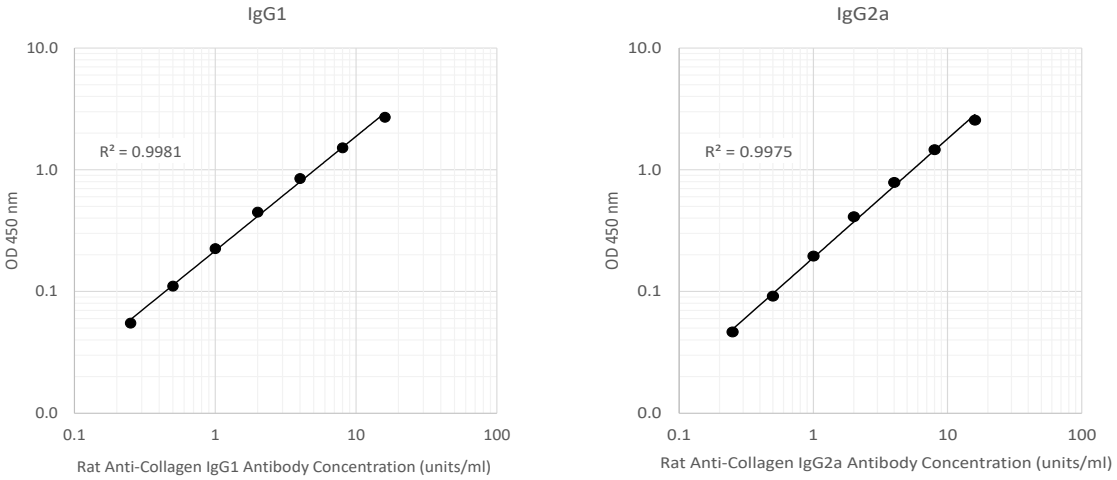
11. **Read Plate:** Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF ANTIBODY TITERS

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

Figure 1 - Typical Standard Curves for the Rat Anti-Collagen IgG1 and IgG2a Antibody Subtype ELISA Kits.



TROUBLESHOOTING

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

REFERENCES

1. L. Mattsson, J. Lorentzen, L. Svelander, A. Bucht, U. Nyman, *et al.*, Immunization with alum-collagen II complex suppresses the development of collagen-induced arthritis in rats by deviating the immune response. *Scand J Immunol* **46**, 619-24 (1997).
2. S. Firth, K. Morgan, H. Evans, P. Holt, IgG subclasses in collagen-induced arthritis in the rat. *Immunol Lett* **7**, 243-7 (1984).
3. S. Jung, Y. Park, H. Lee, J. Shin, G. Lee, S. Park, *et al.*, TGF-beta-treated antigen presenting cells suppress collagen- induced arthritis through the promotion of Th2 responses. *Exp Mol Med* **42**, 187-94 (2010).

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