Chondrex, Inc.

Mouse Anti-Type II Collagen IgG Subtype Antibody Assay Kit

Catalog # 20311-20315, 20321-20325, 20331-20335, 20351-20355, 20361-20365

For Research Use Only - Not Human or Therapeutic Use

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. In CIA susceptible DBA mice, complement fixable IgG2a and IgG2b subtype autoantibody levels, as well as their epitope specificity, are pertinent in the induction of arthritis. In order to adequately study the pathological roles of antibodies to type II collagen, it is important to determine individual IgG subtype autoantibody levels to mouse type II collagen as well as the heterologous type II collagen used for immunization. On the other hand, C57BL/6 mice, a popular strain for developing transgenic mice, produce IgG2c antibodies instead of IgG2a antibodies due to its MHC class I haplotype (H-2b). Moreover, these mice can only develop CIA when immunized with chick type II collagen. Therefore, serum IgG2c autoantibody levels against mouse type II collagen and chick type II collagen should be evaluated when using C57BL/6 mice for the CIA model.

Chondrex, Inc. provides Mouse Anti-Collagen IgG Subtype (IgG1, IgG2a, IgG2b, IgG2c, and IgG3) Assay Kits for further detailed analysis of antibodies in mouse sera from the mouse CIA model. These kits can also be used to determine antibodies to type I collagen (chick, bovine, porcine, human, and mouse) upon request. Please contact Customer Service (contactus@chondrex.com) for more information on custom type I collagen-coated plates.

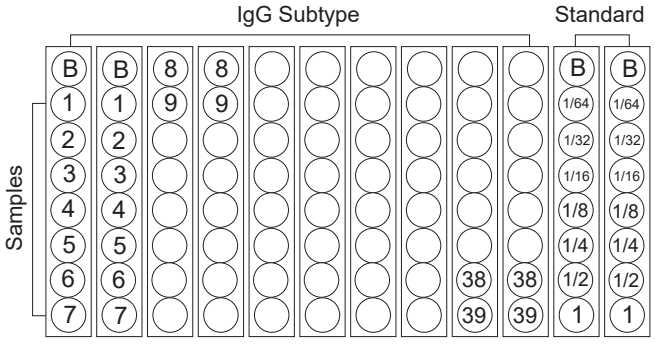
Note: Depending on the IgG subtypes and recognizing epitopes, the antibody-antigen affinity varies significantly among individual antibodies and serum samples from individual animals. Therefore, note the total IgG concentration calculated as the sum of individual IgG subtypes might not perfectly match the total IgG levels determined by our Mouse Anti-Collagen IgG Assay Kit.

Species (Type II Collagen Color Coding)	IgG1 Catalog #	IgG2a Catalog #	IgG2b Catalog #	IgG2c Catalog #	IgG3 Catalog #
Chick (Yellow)	20311	20312	20313	20315	20314
Bovine (Green)	20321	20322	20323	20325	20324
Porcine (Pink)	20331	20332	20333	20335	20334
Human (Blue)	20351	20352	20353	20355	20354
Mouse (Orange)	20361	20362	20363	20365	20364
Standard (Red)	---	---	---	---	---

ELISA Kit for Single IgG Subtype Assay

Figure 1 shows an example of an 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.



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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody IgG1, IgG2a, IgG2b, IgG2c, or IgG3	1 vial	10 ng (IgG3: 100 ng)	-20°C
Secondary Antibody (Peroxidase-Conjugated Anti-Mouse IgG1, IgG2a, IgG2b, IgG2c, or IgG3)	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
OPD (90021)	2 vials	Lyophilized	-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

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 with 1.0 ml of Sample/Standard Dilution Buffer (Solution B) to make the highest standard concentration - labeled "1" in the standard columns of Figure 1. Prepare serial dilutions of the 1X standard by mixing 250 µl of the 1X standard with 250 µl of Solution B - labeled "1/2" in the standard columns of Figure 1. Then repeat this procedure to make a total of six serial dilutions of standard. The 1X standard may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.

   Typical standard dilution: IgG1, IgG2a, IgG2b, or IgG2c: 10, 5, 2.5, 1.25, 0.63, 0.31, and 0.16 ng/ml
   IgG3: 100, 50, 25, 12.5, 6.3, 3.1, and 1.6 ng/ml

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4. **Prepare Sample Dilutions**: Centrifuge serum samples at 10,000 rpm for 3 minutes at room temperature to remove any insoluble materials and lipids. The dilution range for samples may vary depending upon the immunizing antigen, dose, and frequency. IgG subtype antibody contents vary significantly among individual samples. For instance, sera from arthritic mice may contain higher levels of IgG2a or IgG2c followed by IgG1, IgG2b, and IgG3. The following are recommended starting dilutions: samples for IgG1 - 1:2000, IgG2a or IgG2c - 1:10,000, and IgG2b - 1:2000. Optimal dilution will need to be determined for individual samples. For example: Add 10 µl of sample into 990 µl of Solution B (1:100). Keep this at –20°C as a stock solution. Then, dilute 50 µl of the stock solution into 1.95 ml of Solution B (1:4,000).

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 specific to the individual IgG subtype in 10 ml of 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 Chromogen Dilution Buffer just prior to use. Add 100 µl of the OPD solution to all wells immediately after washing the plate. Incubate at room temperature for 30 minutes.

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.

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CALCULATION OF ANTIBODY TITERS

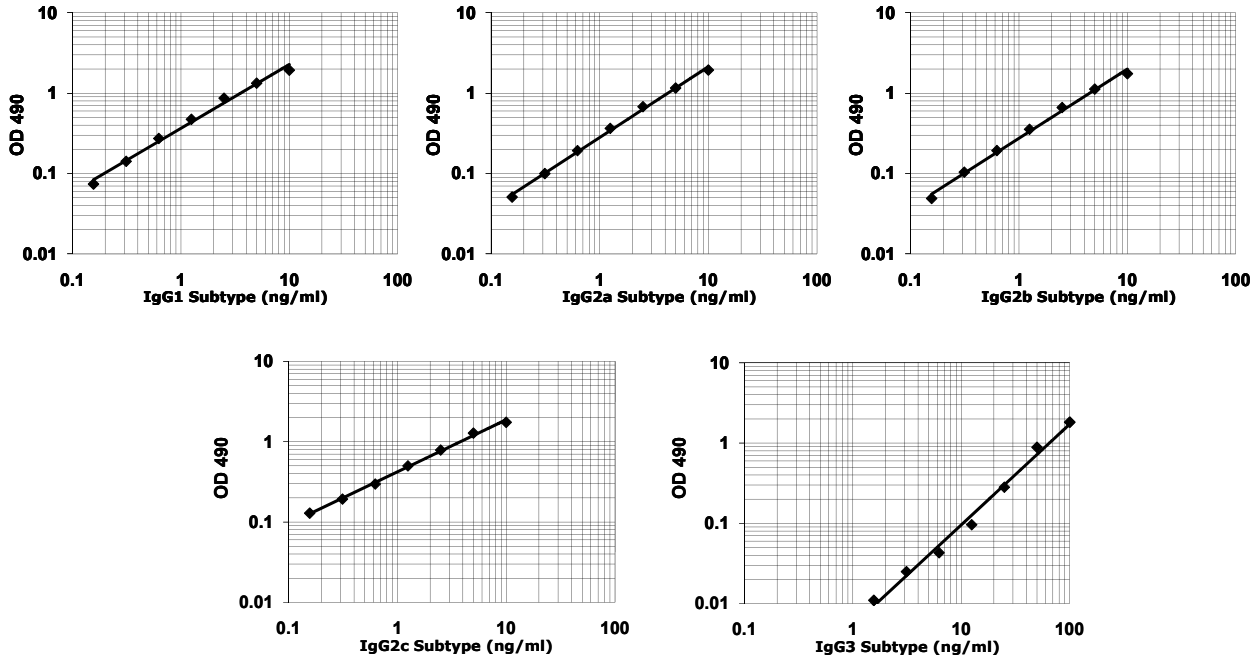
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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

3. Plot the OD values of standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 2 shows an example of standard curves of IgG1, IgG2a, IgG2b, IgG2c, and IgG3 subtype antibodies.

4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution ratio to obtain the antibody concentration (ng/ml) in original sample specimens.

Figure 2 - IgG1, IgG2a, IgG2b, IgG2c, and IgG3 antibody standard curves



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