Chondrex, Inc.

Human/Monkey Anti-Type V Collagen IgG Assay Kit

Catalog # 2082, 2085, 20851

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Human sera, especially from patients with autoimmune diseases, yield high background levels in ELISA systems. These non-specific reactions are caused by adhesive immunoglobulins in human serum, which strongly adhere to plastic surfaces by hydrophobic binding. Furthermore, common blocking agents such as bovine serum albumin (BSA) and Tween 20, are incapable of blocking these non-specific reactions, resulting in false positive reactions. Moreover, these false positive reactions are mistakenly considered as real antibody-antigen reactions. In order to obtain the real value of the antigen-antibody reaction, it is critical to 1) choose proper blocking agents which effectively block these kinds of non-specific reactions, 2) determine unique non-specific background values of individual samples using antigen-non-coated wells and 3) subtract the background value from the value determined in antigen-coated wells. In addition, it is also important to determine the non-specific reactions caused by the secondary antibody. Chondrex Inc.'s ELISA system incorporates original blocking agents that inhibit the hydrophobic binding of these serum components onto plastic surfaces and is designed to determine the background values of individual samples using antigen-non-coated wells.

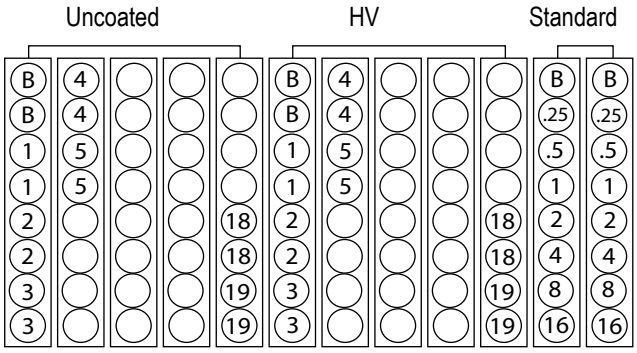
Species	Type V Collagen Color Coding - Catalog #
Bovine Amnion	(BV) Green - 2082
Human Amnion	(HV) Blue - 2085
Human Placenta	(HVP) Silver - 20851
Uncoated	Clear
Standard	Red

Type V collagen is a minor collagen component that forms interstitial collagen fibrils with type I collagen, and plays a critical role in the regulation of fibrillogenesis. Type V collagen from most tissues consists of two α1(V) chains and one α2(V) chain, $[\alpha1(V)]_2\alpha2(V)$, whereas the placenta contains two subtypes of type V collagen, $[\alpha1(V)]_2\alpha2(V)$ and α1(V)α2(V)α3(V). Immune reactions to type V collagen may play a role in graft rejection following lung transplant. For studying the possible involvement of type V collagen immunity in organ allograft rejection in both *in vitro* and *in vivo* experiments, Chondrex Inc. provides type V collagen-coated ELISA plates (wells) in addition to uncoated wells for use as a blank (see table above). This ELISA kit contains enough materials to run two plates on two separate occasions (see assay procedure) and may be used for monkey sera as well as human sera.

Standard ELISA Kit with One Species of Type V Collagen

Figure 1 shows a standard ELISA kit consisting of five 8-well strips which are uncoated and serve as a control for background levels of individual samples, five 8-well strips coated with one species of type V collagen to determine specific antibody levels, and two 8-well strips for reference standards. "B" represents blank wells to determine non-specific reactions caused by the secondary antibody. Standards and samples are run in duplicate.

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



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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody	1 vial	1.1 ml, 16 units/ml	-20°C
Secondary Antibody (Biotin-Conjugated Goat Anti-Human IgG Immunoglobulin Polyclonal Antibody)	2 vials	50 µl, lyophilized	-20°C
Solution A - Blocking Buffer	1 bottle	20 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
Solution D - Streptavidin Peroxidase Dilution Buffer	1 bottle	20 ml	-20°C
Streptavidin Peroxidase	2 vials	50 µl	-20°C
OPD	2 vials	Lyophilized	-20°C
Chromogen Dilution Buffer	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 ml	-20°C
Wash Buffer, 20X	2 bottles	50 ml	-20°C
Type V Collagen-Coated 8-Well Strips	10 each	8-well strips	-20°C
Uncoated 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: Warm up all buffers to room temperature before use.

Note 3: Partially used reagents may be kept at –20°C.

Note 4: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipette, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to avoid the edge effect.

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 at room temperature for 1 hour.

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3. **Prepare Standard Dilutions**: Undiluted standard is 16 units/ml. Serially dilute the standard with Sample/Standard Dilution Buffer (Solution B). For example, mix 250 µl of 16 units/ml standard with an equal volume of Solution B to make a 8 units/ml . Then repeat it five more times for 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**: Add 10 µl of human serum sample to 990 µl of Solution B (1:100), and keep it as a stock solution for future assays. Then, further dilute the sample with Solution B depending on the antibody levels. If necessary, dilute the samples further with Solution B, 1:200-1:1000.

   Note 1: Human serum samples generally contain high lipid levels. In order to avoid non-specific reactions caused by lipids, separate lipids by centrifuging samples at 10,000 rpm for 5 minutes using a tabletop centrifuge, then use serum (the bottom layer) for the assays

   Note 2: It is recommend that a preliminary assay using various dilutions of sera (1:200, 1:500, 1:1,000) in order to determine the optimal dilution of your samples, especially before assaying 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 collagen coated and uncoated 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. **Add Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

11. **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.*

12. **OPD**: Dissolve one vial of OPD in 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of OPD solution to all wells immediately after washing the plate and incubate for 30 minutes at room temperature.

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

14. **Read Plate**: Read the OD values at 490 nm (a 630 nm filter can be used as a reference). If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

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CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the standards, blanks (B) and test samples in uncoated wells and collagen coated wells.

2. Subtract the blank (B) values from the averaged OD values of the standards and test samples in uncoated wells and collagen coated wells.

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

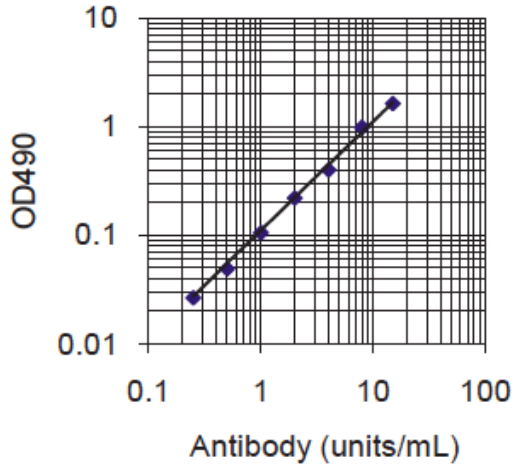
3. Subtract the OD values of samples tested in uncoated wells (background values) from their counterpart OD values in collagen coated wells from step 2 to eliminate values associated with non-specific reactions.

4. Plot the OD values of standards against the units/ml of antibody standard. Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is from 0.25 to 16 units/ml.

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

   Note: 100 units is approximately 1 μg IgG antibody/ml.

Figure 2 - A typical standard curve



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