Version 15-04/24

User's Manual

Human/Monkey Anti-Type I and Type II Collagen IgG Antibody ELISA Kit

REF **DEIA11773**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Assay kit to quantify human/monkey anti-collagen antibodies.

Principles of Testing

Human serum, especially from patients with autoimmune diseases, contain high levels of immunoreactive components, which yield high background levels in ELISA systems. These non-specific reactions are caused by adhesive immunoglobulins in human serum which so strongly adhere to plastic surfaces by hydrophilic binding that blocking agents such as bovine serum albumin (BSA) and Tween 20 are not capable of blocking these non-specific reactions at all. False positive reactions caused by the serum samples themselves are usually overlooked and are considered real antibody-antigen reactions in many cases, even now. In order to obtain the real values of antigenantibody reactions, it is critical 1) to choose proper blocking agents which block these kinds of non-specific reactions effectively, 2) to determine the unique non-specific background value of each individual sample using antigen-non-coated wells, and 3) to subtract that background value from the corresponding value determined in antigen-coated wells. In addition, it is important to determine the non-specific reactions caused by the secondary antibody as well. The ELISA system incorporates unique blocking agents that inhibit the hydrophobic binding of these serum components onto plastic surfaces and are designed to determine the background values of individual samples using antigen-non-coated wells.

In general, antibodies to collagen in human serum are highly specific to heterologous collagen such as bovine or chick type I and/or type II collagen and cross-react to other species and types of collagen including human collagen, regardless of disease. For example, even serum antibodies from healthy normal controls react to various species and types of collagen. To determine the human serum antibody levels specific to the desired antigen we provides various species of type I and type II collagen-coated strips as well as uncoated wells. This ELISA kit contains enough materials to run two plates on two separate occasions and may be used for monkey serum as well as human serum.

Reagents And Materials Provided

1. Standard IgG Antibody, 1 vial 1.1 ml, 16 units/ml -20°C
2. Secondary Antibody (Biotin-Conjugated Goat Anti-Human IgG), 2 vials Lyophilized, -20°C
3. Solution A - Blocking Buffer, 1 bottle 20 ml, -20°C
4. Solution B - Sample/Standard Dilution Buffer, 1 bottle 50 ml, -20°C
5. Solution C - Secondary Antibody Dilution Buffer, 1 bottle 20 ml, -20°C
6. Solution D - Streptavidin Peroxidase Dilution Buffer, 1 bottle 20 ml, -20°C
7. Streptavidin Peroxidase, 2 vials 50 ml, -20°C
8. TMB, 2 vials 0.2 ml, -20°C
9. Chromogen Dilution Buffer, 1 bottle 20 ml, -20°C
10. Stop Solution - 2N Sulfuric Acid, 1 bottle 10 ml, -20°C
11. Wash Buffer, 20×, 2 bottles 50 ml, -20°C
12. Type I or Type II Collagen-Coated 8-Well Strips, 10 each 8-well strips, -20°C

13. Uncoated 8-Well Strips, 10 each 8-well strips, -20°C
14. Reference Standard Strips (two strips per run), 4 each 8-well strips, -20°C

Storage

-20°C

Assay Procedure

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, 20× 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.

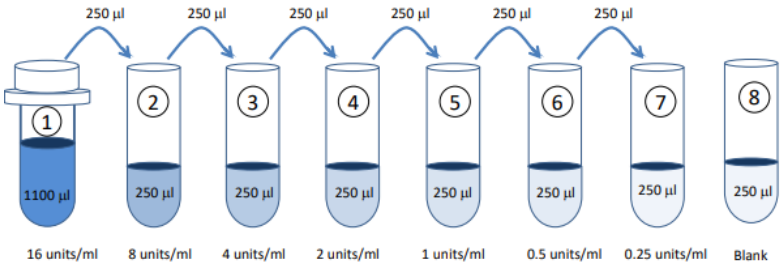
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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^{0}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 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: The undiluted standard stock solution is 16 units/ml. 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 CD recommends making fresh serial dilutions for each assay.



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 1:100 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 necessary,

dilute the samples further with Solution B, 1:200 - 1:1000.

4. Dilute Wash Buffer: Dilute 50 ml of 20× wash buffer in 950 ml of distilled water (1× wash buffer). Wash the plate with 1× 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 1× 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: Dissolve one vial of secondary antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial in 50 µl of Solution C and dilute accordingly. 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. Add Streptavidin Peroxidase: Dilute one vial of streptavidin peroxidase in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

Strip #	Streptavidin Peroxidase (µl)	Solution D (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

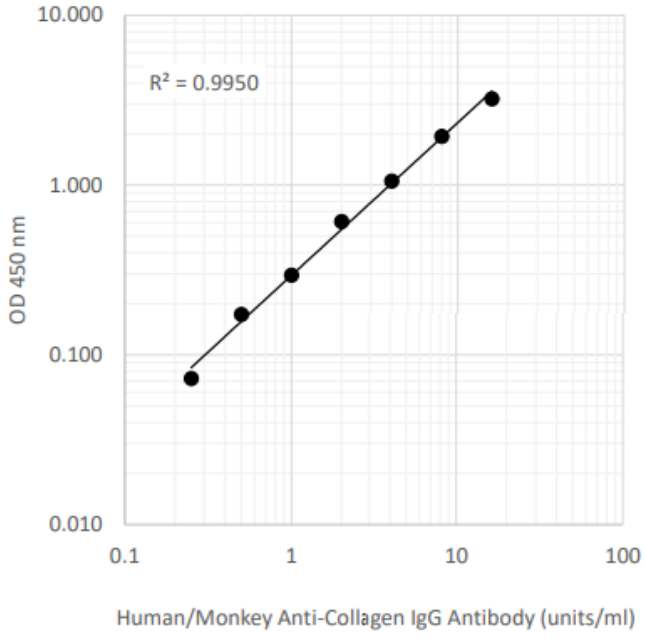
10. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. Do not allow the plate to dry out.
11. TMB: Dilute one vial of TMB in 10 ml of Chromogen 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

12. Stop: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.
13. 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, reassay the samples at a higher dilution. A 630 nm filter can be used as a reference.

Calculation

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



Detection Range

16 units/ml to 0.25 units/ml

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com