Human anti-Bacteria & Toxins Antibody Assay Kits

Catalog # 6113 - 6128

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

A growing body of research has indicated an association between intestinal bacteria and autoimmune diseases. More specifically, dysbiosis or imbalance of intestinal bacterial flora may contribute to the pathogenesis of Rheumatoid Arthritis (RA), as indicated by many studies on intestinal microbes (1-5). Furthermore, recent studies have also suggested a possible link between RA and periodontal diseases caused by Porphyromonas gingivalis (*P. gingivalis*) (6-8) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) (9). Normally, intestinal bacteria do not affect the host's health, but under certain conditions, may overcome the host's defenses and exert pathogenic effects. Examples of these include immunosenescence (10-13), gastrointestinal disorders such as constipation (14) and diarrhea (15), or other events such as physical and psychological stress (16, 17). It is therefore important to take into consideration these risk factors when studying the etiology of autoimmune diseases. Intestinal bacterial imbalance may increase the levels of pathogenic substances in the gastrointestinal lumen and high mucosal permeability may increase translocation of potential pathogenic agents into the circulatory system. Consequently, pathogens, which may overwhelm the host's defense functions and cause chronic health problems that will evolve into autoimmune disorders (18, 19).

To facilitate and promote studies that determine immune responses to environmental agents in humans, Chondrex, Inc. provides ELISA kits for assaying human serum antibodies against a variety of potential pathogenic and non-pathogenic environmental agents, of which all humans may be universally exposed to during their lifetime. For further requests or consultation, please contact us at support@chondrex.com.

These ELISA kits employ ChonBlock™ (Catalog # 9068 and Catalog # 90681) assay buffers. ChonBlock™ eliminates non-specific reactions involved in the indirect ELISA, especially false positive reactions caused by hydrophobic binding of immunoglobulin in sample specimens to ELISA plates as reported in detail (20-22).

Catalog # and Plate Color Coding

Antigens	IgG	IgA
E. coli (O111:B4) Lipopolysaccharide (LPS)	Red - 6113	Red - 6114
E. coli (O111:B4)	Yellow - 6115	Yellow - 6116
P. gingivalis LPS	Pink - 6117	Pink - 6118
P. gingivalis	Orange - 6119	Orange - 6120
Lactobacillus casei	Purple - 6121	Purple - 6122
Peptidoglycan Polysaccharide (PG-PS)	Blue - 6123	Blue - 6124
Salmonella	Green - 6125	Green - 6126
Yeast Extract	Gray - 6127	Gray - 6128

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody IgG or IgA	1 vial	32 units, lyophilized	-20°C
IgG or IgA Secondary Antibody (peroxidase-conjugated goat polyclonal antibodies) (61133 or 61143)	2 vials	50 µl/vial	-20°C
Solution A - Blocking Buffer (61026)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (61027)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (61025)	1 bottle	20 ml	-20°C
TMB Solution (90023)	2 vials	0.2 ml/vial	-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
Antigen coated 8-Well Strips	12 each	8-well strips	-20°C

An antigen uncoated plate (Catalog #9026) is not included. Please contact us at support@chondrex.com to place an order.

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 pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

PLATE MAPPING

Map the plate based on the number of samples and sample dilution. For example, if sample dilution is more than 1:1,000, it is not necessary to run antigen uncoated wells (Figure 1a), but if sample dilution is less than 1:1,000, it may be necessary to run antigen uncoated wells to determine the background noise (BG) reaction OD values of individual samples (Figure 1b).

Figure 1a - Standard layout of antigen coated plate.

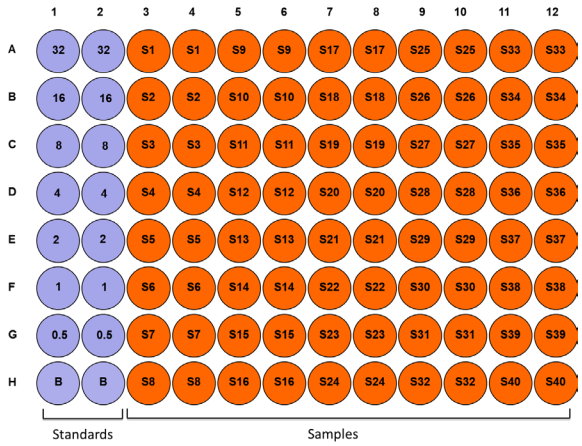
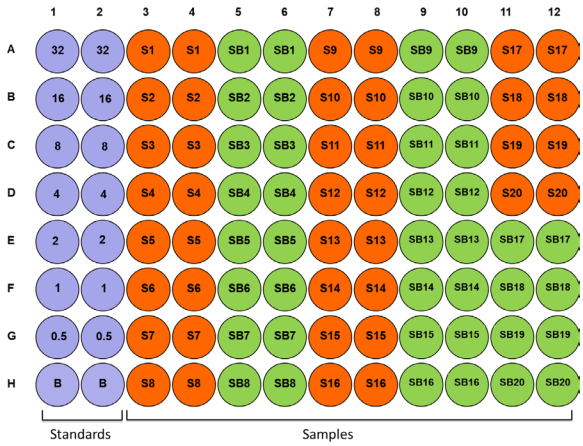


Figure 1b - Standard layout of antigen coated and uncoated plate.



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ASSAY PROCEDURE

1. **Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer).

2. **Add Blocking Buffer:** Add 100 µl of the Blocking Buffer (Solution A) to each well and incubate for 1 hour at room temperature

   Note: If a sample with a dilution of 1:1,000 or less is assayed, antigen non-coated strips should be used. Solution A must be added to the non-coated wells without prior washing because any contaminants in the vessel containing the washing buffer will bind to the strips. For example, add 100 µl of Solution A to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1) as mapped out in Figure 1b. Incubate for 1 hour at room temperature.

3. **Prepare Standard Dilutions:** Dissolve one vial of standard (32 units/vial) with 1 ml Standard/Sample dilution buffer (Solution B) to make a 32 units/ml stock standard solution. Prepare standard serial dilutions by mixing 250 µl of the stock standard solution with 250 µl of Solution B (16 units/ml). Repeat this procedure to make 8, 4, 2, 1, and 0.5 units/ml standard solutions for a total of 7 serial standard dilutions. Keep the remaining 32 units/ml stock standard solution at -20°C for future assays.

4. **Prepare Sample Dilutions:** Add 10 µl of mouse 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. For example, take 50 µl of the sample stock solution and mix with 450 µl of solution B to make a 1:1,000 dilution. If it is necessary to assay antibodies at a low dilution of less than 1:1,000 due to low antibody levels, antigen uncoated control strips will be necessary. Please contact support@chondrex.com.

   Note: Chondrex, Inc. recommends running a preliminary assay using various dilutions of sera (1:1,000, 1:4,000, 1:16,000) 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 wells in duplicate according to the desired layout (Figure 1a or Figure 1b). Incubate at room temperature for 2 hours.

   Note: If a sample with a dilution of 1:1.000 or less is assayed, add 100 µl of the diluted samples to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1) as mapped out in Figure 1b.

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**: Dilute one vial of Secondary Antibody with 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 1 hour.

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 TMB**: Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of the TMB solution to all wells immediately after washing the plate and incubate at room temperature for 25 minutes.

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

12. **Read Plate**: Read the OD values at 450 nm (a 630 nm filter can be used as a reference) within 5 minutes after added Stop Solution. If the OD values of the 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 blanks (BL), test samples and standards.
2. Subtract the blank values from the averaged OD values of the test samples and standards respectively.

   NOTE: If the antigen uncoated wells were used, subtract the OD values of samples tested in uncoated wells (background values) from their counterpart OD values in antigen coated wells from step 2 to eliminate values associated with non-specific reactions.
3. Plot the OD values of standards against the unit/ml of standard. A log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is from 0.5 to 32 units/ml.
4. 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 original sample specimens.

Figure 2 - Typical standard curves for human anti-bacteria and its toxins antibody ELISA.

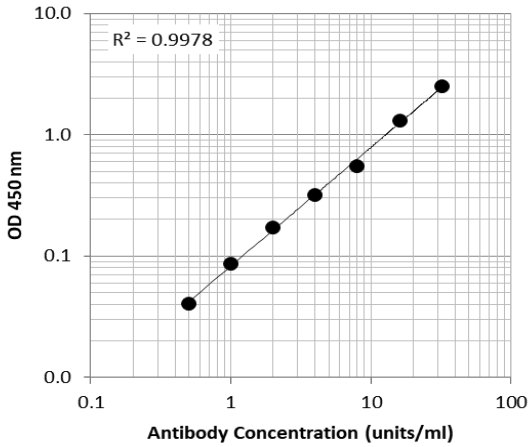

Table 1 - Reproducibility data for Anti-*E. coli* (O111:B4) LPS Antibody Assay Kit

	IgG (6113)			IgA (6114)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	3.7	6.3	6.5	9.8	9.3	4.2
Intra-Assay CV (%)	3.2	6.2	7.2	8.8	5.0	3.
Spiking Test*	97%	109%	95%	103%	97%	94%

Table 2 - Reproducibility data for Anti-*E. coli* (O111:B4) Antibody Assay Kit

	IgG (6115)			IgA (6116)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	9.8	8.0	6.0	3.8	2.0	10.0
Intra-Assay CV (%)	4.0	4.7	1.2	1.1	3.8	5.3
Spiking Test*	95%	107%	90%	88%	94%	91%

* Standard was added with known amounts of antibody and then diluted with Sample/Standard Dilution Buffer to assay antibodies by ELISA.

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Table 3 - Reproducibility data for Anti-*P. gingivalis* LPS Antibody Assay Kit

	IgG (6117)			IgA (6118)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	9.0	5.8	7.7	9.9	4.5	6.3
Intra-Assay CV (%)	2.5	8.8	2.8	8.9	1.1	1.9
Spiking Test*	94%	96%	95%	105%	108%	105%

Table 4 - Reproducibility data for Anti-*P. gingivalis* Antibody Assay Kit

	IgG (6119)			IgA (6120)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	6.0	6.3	9.0	10.9	1.5	5.8
Intra-Assay CV (%)	7.3	1.9	2.8	3.3	5.3	9.0
Spiking Test*	95%	95%	110%	91%	89%	91%

Table 5 - Reproducibility data for Anti-*Lactobacillus* Antibody Assay Kit

	IgG (6121)			IgA (6122)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	9.2	4.4	7.3	6.0	9.2	9.4
Intra-Assay CV (%)	3.9	7.3	1.3	6.0	2.6	0.6
Spiking Test*	92%	94%	98%	96%	102%	93%

Table 6 - Reproducibility data for Anti-PG-PS Antibody Assay Kit

	IgG (6123)			IgA (6124)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	6.1	1.7	8.9	7.5	9.7	4.3
Intra-Assay CV (%)	6.8	1.2	8.9	7.3	3.5	6.5
Spiking Test*	108%	101%	95%	94%	96%	96%

Table 7 - Reproducibility data for Anti-Salmonella Antibody Assay Kit

	IgG (6125)			IgA (6126)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	4.6	4.0	7.8	8.4	4.8	4.0
Intra-Assay CV (%)	4.2	5.0	8.0	4.9	3.6	2.9
Spiking Test*	90%	91%	98%	92%	91%	93%

Table 8 - Reproducibility data for Anti-Yeast Extract Antibody Assay Kit

	IgG (6127)			IgA (6128)		
Test At	**1 unit/ml**	**4 units/ml**	**16 units/ml**	**1 unit/ml**	**4 units/ml**	**16 units/ml**
Inter-Assay CV (%)	6.5	6.9	8.3	9.7	3.4	9.8
Intra-Assay CV (%)	7.5	6.1	1.9	6.6	9.0	5.8
Spiking Test*	110%	103%	104%	95%	102%	93%

* Standard was added with known amounts of antibody and then diluted with Sample/Standard Dilution Buffer to assay antibodies by ELISA.

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