Mouse Anti-*Escherichia coli* Lipopolysaccharide (O111:B4) Antibody ELISA Kits

Catalog # 6106, 6107, 6110, 6111, and 6112

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kits to quantify mouse anti-*E. coli* LPS IgG/IgG1/IgG2a/IgG2b/IgG3 antibodies
	6106: Mouse Anti-*E. coli* LPS IgG Antibody ELISA Kit
	6107: Mouse Anti-*E. coli* LPS IgG1 Antibody ELISA Kit
	6110: Mouse Anti-*E. coli* LPS IgG2a Antibody ELISA Kit
	6111: Mouse Anti-*E. coli* LPS IgG2b Antibody ELISA Kit
	6112: Mouse Anti-*E. coli* LPS IgG3 Antibody ELISA Kit
FORMAT:	Pre-coated 96-well ELISA Plate with non-removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	6106/6107/6110/6111 : 100 ng/ml to 1.6 ng/ml
	6112 : 500 ng/ml to 8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) diluted samples/kit and up to 20 (duplicate) low dilution samples/kit
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	6106: Intra-assay (0.9-7%)/Inter-assay (2-11.7%)/Spiking Test (93-106%)
	6107: Intra-assay (3.6-8.8%)/Inter-assay (9.3-13.1%)/Spiking Test (106-108%)
	6110: Intra-assay (1-3.1%)/Inter-assay (2.6-6.1%)/Spiking Test (88-99%)
	6111: Intra-assay (3.1-6.9%)/Inter-assay (1.9-9.8%)/Spiking Test (106-115%)
	6112: Intra-assay (0.1-5.8%)/Inter-assay (3.9-9.1%)/Spiking Test (91-96%)
NOTES:	N/A

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

Mouse Anti-*Escherichia coli* Lipopolysaccharide (O111:B4) Antibody ELISA Kits

Catalog # 6106, 6107, 6110, 6111, and 6112

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Recent studies indicate environmental factors, especially intestinal microbiota and their toxins, may play pathogenic roles in autoimmune diseases such as rheumatoid arthritis (RA) (1-7), inflammatory bowel diseases (IBDs) (8, 9), systemic lupus erythematosus (SLE) (10), and other chronic disorders (11-13). It is possible that increased translocation of bacteria and bacterial toxins associated with high mucosal permeability and low immune function may be the primary and common pathogenesis of these autoimmune disorders (14). Mice are ideal experimental animals to test this hypothesis due to the variety in genetic backgrounds, strains, and housing conditions, such as germ free, gnotobiotic, specific pathogen free, and conventional, all of which can affect susceptibility to potential pathogens. These differences in susceptibility may be attributed to the maturity of the immune system, depending on the environment.

To facilitate and promote studies that determine immune responses to environmental agents, Chondrex. Inc. provides mouse antibody ELISA kits against *Escherichia coli* O111:B4 lipopolysaccharide (*E. coli* LPS). In addition, Chondrex, Inc. also provides mouse antibody ELISA kits against *E. coli* O111:B4 (Cat # 6206, 6207, 6209 - 6212), staphylococcal enterotoxin A (Cat # 6218 - 6221), staphylococcal enterotoxin B (Cat # 6214 - 6217), *Porphyromonas gingivalis* (Cat # 6225 - 6227), and *Porphyromonas gingivalis* LPS (Cat # 6222 - 6224). For further requests or consultation, please contact us at support@chondrex.com.

Mouse anti-*E. coli* LPS IgG Antibody ELISA Kit (Cat # 6106)
Mouse anti-*E. coli* LPS IgG1 Antibody ELISA Kit (Cat # 6107)
Mouse anti-*E. coli* LPS IgG2a Antibody ELISA Kit (Cat # 6110)
Mouse anti-*E. coli* LPS IgG2b Antibody ELISA Kit (Cat # 6111)
Mouse anti-*E. coli* LPS IgG3 Antibody ELISA Kit (Cat # 6112)

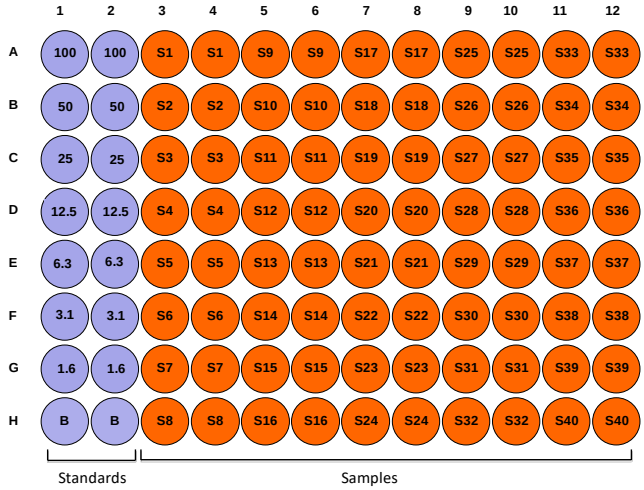
KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard Antibody	IgG (61066) IgG1 (61076) IgG2a (61106) IgG2b (61116) IgG3 (61121)	1 vial	IgG/IgG1/IgG2a/IgG2b 100 ng, lyophilized IgG3 500 ng, lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (61062) IgG1 (61072) IgG2a (61102) IgG2b (61112) IgG3 (61123)	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
LPS (from *E. coli* O111:B4)-coated 8-Well Strips (Red)		12 each	8-well strips	-20°C

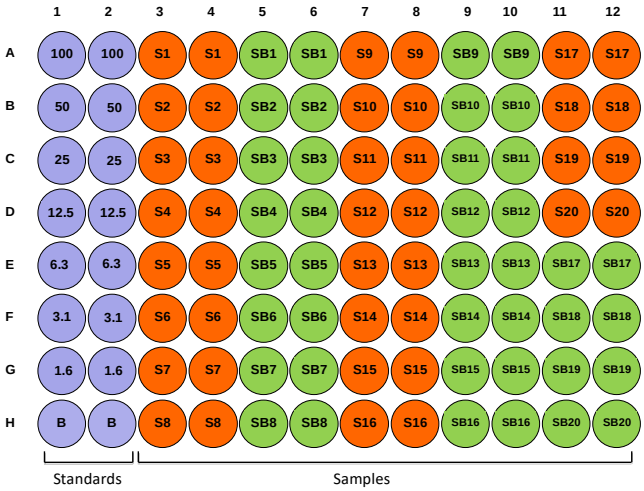
PLATE MAPPING

Map the plate based on the number of samples and sample dilution. For example, if sample dilution is higher than 1:100, it is not necessary to run antigen un-coated wells, but if sample dilution is less than 1:100, it may be necessary to run antigen uncoated wells to determine the background noise reaction OD values of individual samples. An antigen uncoated plate (Catalog # 9026) for lower sample dilutions is not included. Please contact support@chondrex.com to place an order.

Standard Layout of Antigen Coated Plate.



Standard Layout of Antigen Coated and Uncoated Plate.



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.

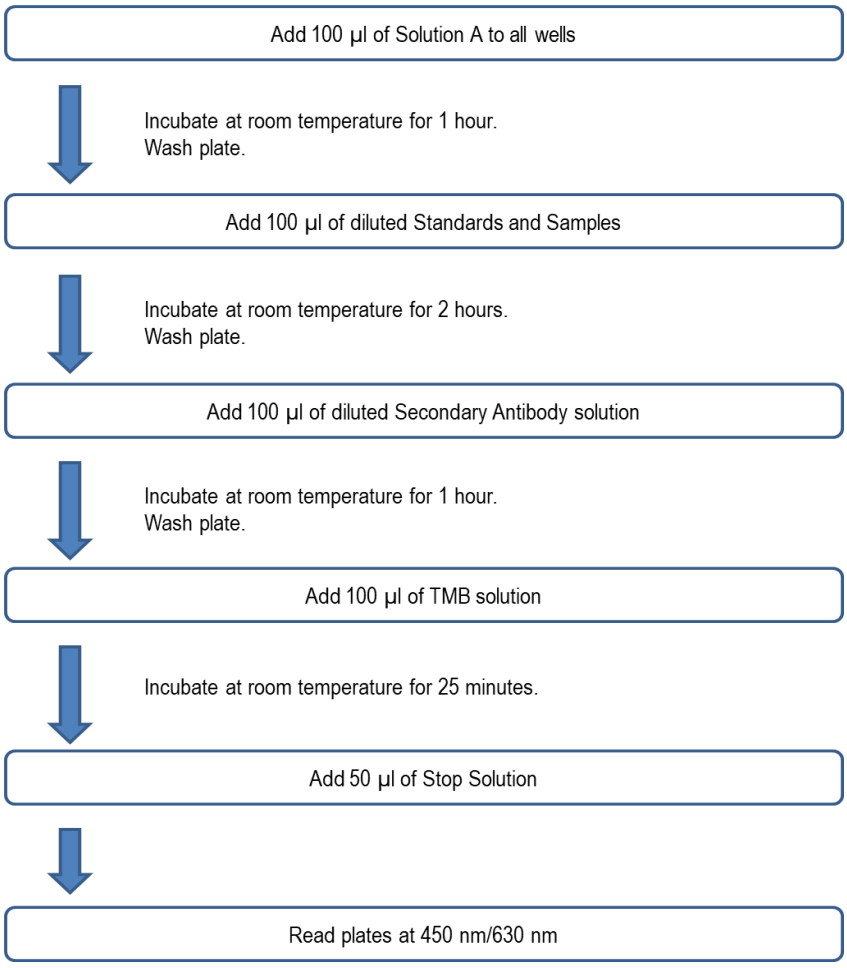
NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

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 -20ºC 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

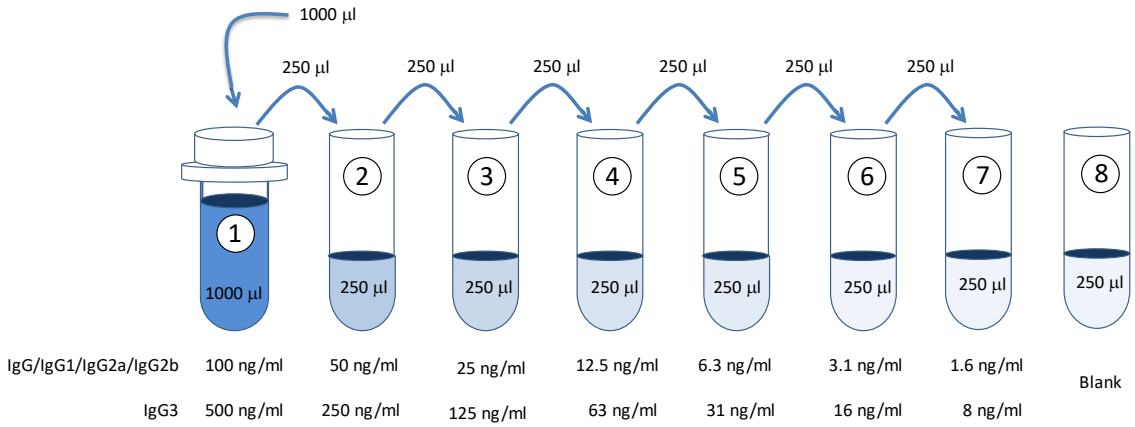


ASSAY PROCEDURE

1. **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 1:100 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). Incubate for 1 hour at room temperature.

2. **Prepare Standard Dilutions**: Dissolve one vial of standard with 1 ml of Sample/Standard Dilution Buffer (Solution B) to make the highest standard concentration - labeled "1". Prepare serial dilutions by mixing 250 µl of the 1X standard with 250 µl of Solution B - labeled "2". Then repeat this procedure to make five additional serial dilutions of standard. The 1X standard may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **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 250 µl of the sample stock solution and mix with 250 µl of Solution B to make a 1:200 dilution. If it is necessary to assay antibodies at less than 1:100 due to low antibody levels, antigen uncoated control strips will be necessary.

   NOTE: Chondrex, Inc. recommends running a preliminary assay using various dilutions of sera (1:200, 1:1,000, 1:5,000) in order to determine the optimal dilution of your samples, especially before assaying a large number of 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 blot 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 Solution B (blank), standards, and samples to designated wells in duplicate. Incubate at room temperature for 2 hours.

   NOTE: If a sample with a dilution of 1:100 or less is assayed, add 100 µl of the diluted samples to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1).

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 blot 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 solution 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.

Strip #	Secondary Antibody (µl)	Solution C (ml)
2	9	1.8
4	17	3.4
6	25	5.0
8	33	6.6
10	41	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 TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, dilute one vial of TMB with 10 ml of Chromogen Dilution Buffer. The prepared TMB solution cannot be stored for a future assay. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 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**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 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.

CALCULATING RESULTS

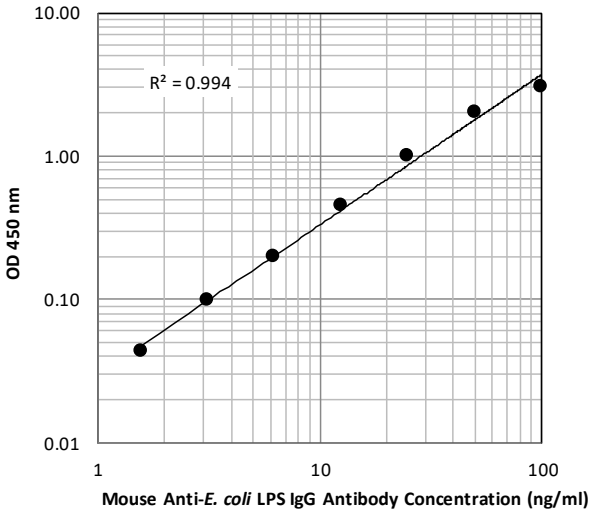
1. Average the duplicate OD values for the standards, blanks (B) and test samples in uncoated wells and coated wells.

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

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

4. Plot the OD values of standards against the ng/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 1.6 to 100 ng/ml. The ng/ml of antibody in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the Mouse Anti-*E. coli* LPS IgG Antibody ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for Mouse Anti-*E. coli* LPS IgG Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	7.0	6.0	0.9
Inter-Assay CV (%)	11.7	4.0	2.0
Spiking Test*	93%	105%	106%

Table 2 - Reproducibility Data for Mouse Anti-*E. coli* LPS IgG1 Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	8.8	4.5	3.6
Inter-Assay CV (%)	9.7	13.1	9.3
Spiking Test*	108%	107%	106%

Table 3 - Reproducibility Data for Mouse Anti-*E. coli* LPS IgG2a Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	2.3	1.0	3.1
Inter-Assay CV (%)	6.1	4.8	2.6
Spiking Test*	98%	99%	88%

Table 4 - Reproducibility Data for Mouse Anti-*E. coli* LPS IgG2b Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	5.4	3.1	6.9
Inter-Assay CV (%)	1.9	9.8	8.1
Spiking Test*	106%	110%	115%

Table 5 - Reproducibility data for Mouse Anti-*E. coli* LPS IgG3 Antibody ELISA Kit

Test	16 ng/ml	63 ng/ml	250 ng/ml
Intra-Assay CV (%)	5.8	4.7	0.1
Inter-Assay CV (%)	9.1	3.9	5.7
Spiking Test*	95%	96%	91%

* Known amounts of anti-*E. coli* LPS antibodies were added to standards and then diluted with Sample/Standard Dilution Buffer (Solution B).

TROUBLESHOOTING

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

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

REFERENCES

1. S. Aoki, K. Yoshikawa, T. Yokoyama, T. Nonogaki, S. Iwasaki, *et al*., Role of Enteric Bacteria in the Pathogenesis of Rheumatoid Arthritis: Evidence for Antibodies to Enterobacterial Common Antigens in Rheumatoid Sera and Synovial Fluids. *Ann Rheum Dis* **55**, 363-9 (1996).

2. I. van der Heijden, B. Wilbrink, I. Tchetverikov, I. Schrijver, L. Schouls, *et al*., Presence of Bacterial DNA and Bacterial Peptidoglycans in Joints of Patients With Rheumatoid Arthritis and Other Arthritides. *Arthritis Rheum* **43**, 593-8 (2000).

3. K. Terato, X. Ye, H. Miyahara, M. Cremer, M. Griffiths, Induction by Chronic Autoimmune Arthritis in DBA/1 Mice by Oral Administration of Type II Collagen and Escherichia Coli Lipopolysaccharide. *Br J Rheumatol* **35**, 828-38 (1996).

4. R. Peltonen, M. Nenonen, T. Helve, O. Hänninen, P. Toivanen, E. Eerola, *et al*., Faecal Microbial Flora and Disease Activity in Rheumatoid Arthritis During a Vegan Diet. *Br J Rheumatol* **36**, 64-8 (1997).

5. P. Toivanen, Normal Intestinal Microbiota in the Aetiopathogenesis of Rheumatoid Arthritis. *Ann Rheum Dis* **62**, 807-11 (2003).

6. J. Vaahtovuo, E. Munukka, M. Korkeamäki, R. Luukkainen, P. Toivanen, Fecal Microbiota in Early Rheumatoid Arthritis. *J Rheumatol* **35**, 1500-5 (2008).

7. C. Edwards, Commensal Gut Bacteria and the Etiopathogenesis of Rheumatoid Arthritis. *J Rheumatol* **35**, 1477-14797 (2008).

8. D. Shi, J. Das, G. Das, Inflammatory Bowel Disease Requires the Interplay Between Innate and Adaptive Immune Signals. *Cell Res* **16**, 70-4 (2006).

9. S. Nell, S. Suerbaum, C. Josenhans, The Impact of the Microbiota on the Pathogenesis of IBD: Lessons From Mouse Infection Models. *Nat Rev Microbiol* **8**, 564-77 (2010).

10. T. Cavallo, N. Granholm, Bacterial Lipopolysaccharide Induces Long-Lasting IgA Deficiency Concurrently With Features of Polyclonal B Cell Activation in Normal and in Lupus-Prone Mice. *Clin Exp Immunol* **84**, 134-8 (1991).

11. W. Penhale, P. Young, The Influence of the Normal Microbial Flora on the Susceptibility of Rats to Experimental Autoimmune Thyroiditis. *Clin Exp Immunol* **72**, 288-92 (1988).

12. M. Murakami, T. Tsubata, R. Shinkura, S. Nisitani, M. Okamoto, *et al*., Oral Administration of Lipopolysaccharides Activates B-1 Cells in the Peritoneal Cavity and Lamina Propria of the Gut and Induces Autoimmune Symptoms in an Autoantibody Transgenic Mouse. *J Exp Med* **180**, 111-21 (1994).

13. M. Nymark, P. Pussinen, A. Tuomainen, C. Forsblom, P. Groop, *et al*., Serum Lipopolysaccharide Activity Is Associated With the Progression of Kidney Disease in Finnish Patients With Type 1 Diabetes. *Diabetes Care* **32**, 1689-93 (2009).

14. K. Terato, C. Do, D. Cutler, T. Waritani, H. Shionoya, Preventing Intense False Positive and Negative Reactions Attributed to the Principle of ELISA to Re-Investigate Antibody Studies in Autoimmune Diseases. *J Immunol Methods* **407**, 15-25 (2014).

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com