Mouse anti-Lipopolysaccharide from *Porphyromonas gingivalis* Antibody ELISA Kits

Catalog # 6222, 6223, and 6224

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-PG LPS IgG/IgM/IgG3 antibodies
	6222: Mouse Anti-PG LPS IgG Antibody ELISA Kit
	6223: Mouse Anti-PG LPS IgM Antibody ELISA Kit
	6224: Mouse Anti-PG LPS IgG3 Antibody ELISA Kit
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	6222/6224: 500 ng/ml to 8 ng/ml
	6223: 100 ng/ml 1.6 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:	1:200 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	6222: Intra-Assay (5.6-9.5%)/Inter-Assay (1.8-5.2%)/Spiking Test (98-105%)
	6223: Intra-Assay (2.7-4.1%)/Inter-Assay (2.9-9.5%)/Spiking Test (90-97%)
	6224: Intra-Assay (3.5-7%)/Inter-Assay (5.7-9.4%)/Spiking Test (98-108%)
NOTES:	N/A

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INTRODUCTION

Recent studies indicate that environmental factors, especially intestinal microbiota and their toxins, may play roles in the development of 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). In fact, germ-free mice fail to develop ankylosing enthesopathy, a naturally occurring joint disease in mice with many parallels to human ankylosing spondylitis (15). Moreover, several studies indicate that increased intestinal mucosal permeability due to stress, surgery, and minor gastrointestinal disorders such as irregularity, may contribute to excess translocation of gut bacteria and their toxins into the body (16-18). These events suggest the pathological importance of intestinal bacteria with regard to autoimmune disorders.

Among the many environmental factors, gram-negative bacteria and their toxins, such as *Porphyromonas gingivalis* (*P. gingivalis*), *Escherichia coli* (*E. coli*), and lipopolysaccharide (LPS), induce inflammation by increasing levels of inflammatory cytokines, and may be involved in various types of chronic autoimmune disorders. In fact, chronic *P. gingivalis* oral infection prior to arthritis induction increases the likelihood of Th17 cell responses, ultimately accelerating collagen-induced arthritis (CIA) development in mice (19). Interestingly, orally administered *P. gingivalis* also perturbs gut microbiomes in CIA mice, affecting the gut immune system and the gut microbiota composition (20). Although intraperitoneal *E. coli*-LPS combined with sub-arthritogenic levels of anti-type II collagen antibodies induces severe arthritis in mice, mice who were instead given LPS from *P. gingivalis* (PG-LPS) failed to develop arthritis (21). Thus, the contribution of these factors in autoimmune disorders requires more studies.

In order to advance these microbiome-related research fields, Chondrex, Inc. provides mouse anti- *P. gingivalis* (33277) and *P. gingivalis* -LPS (33277) antibody ELISA kits to elucidate the host immune response to potential environmental pathogens. For a complete list of available mouse anti-bacteria and bacterial toxins antibody ELISA kits, such as *E. coli* (O111:B4), *E. coli* -LPS (O111:B4), and staphylococcal enterotoxins, please visit www.chondrex.com or contact us at support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard IgG (62221) IgM (62231) IgG3 (62241)	1 vial	Lyophilized IgG/IgG3: 500 ng IgM: 100 ng	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies) IgG (62223) IgM (62233) IgG3 (62243)	2 vials	50 µl	-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 - Detection Antibody Dilution Buffer (61025)	1 bottle	20 ml	-20°C
TMB (90023)	2 vials	0.2 ml	-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
PG-LPS Coated 96-Well ELISA Plate (Pink)	1 each	8-well Strips x12	-20°C

An antigen uncoated plate (Cat # 9026) for lower sample dilutions is not included. Please contact support@chondrex.com to place an order.

IDENTIFICATION OF ANTIGEN-COATED STRIPS

Antigens	Color Coding
E. coli LPS (O111:B4)	Red
E. coli	Yellow
P. gingivalis LPS (PG-LPS)	Pink
P. gingivalis (PG)	Orange

ASSAY OUTLINE

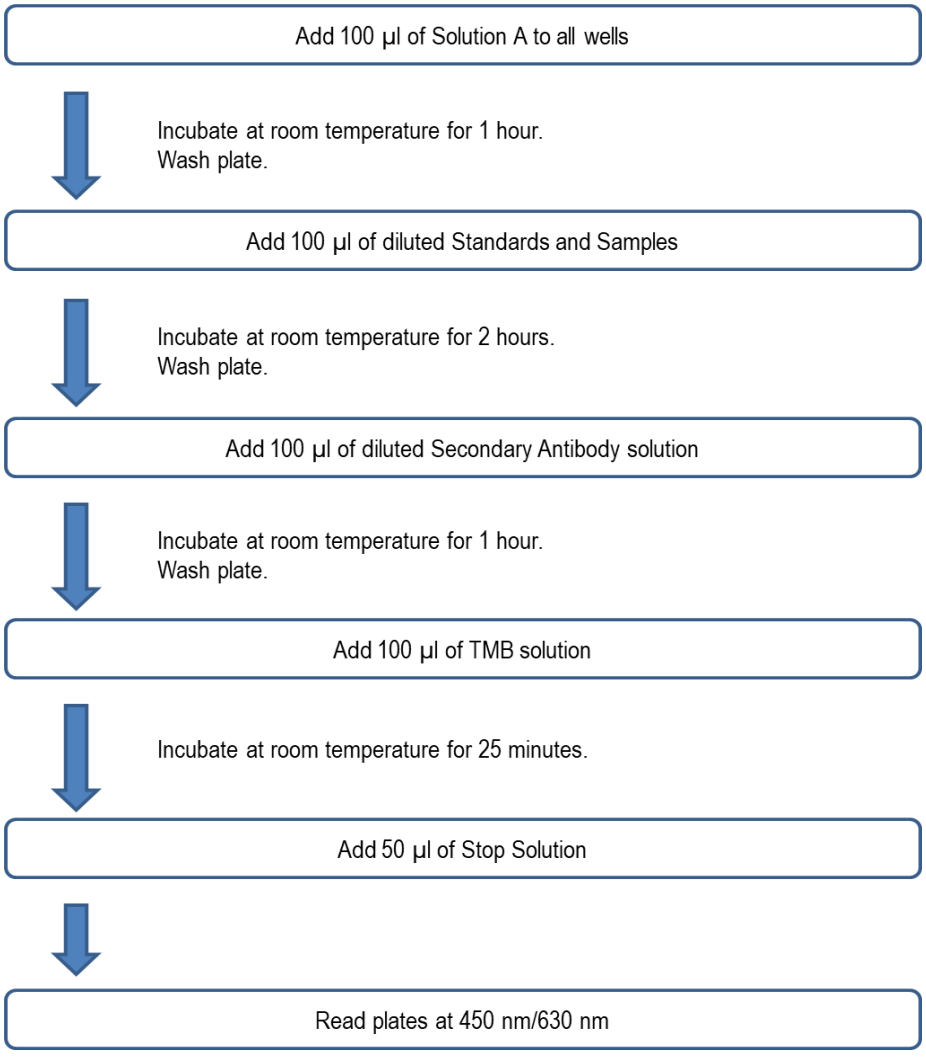

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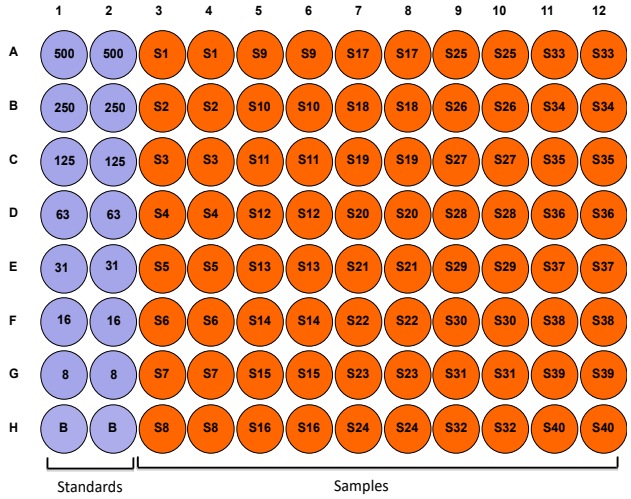
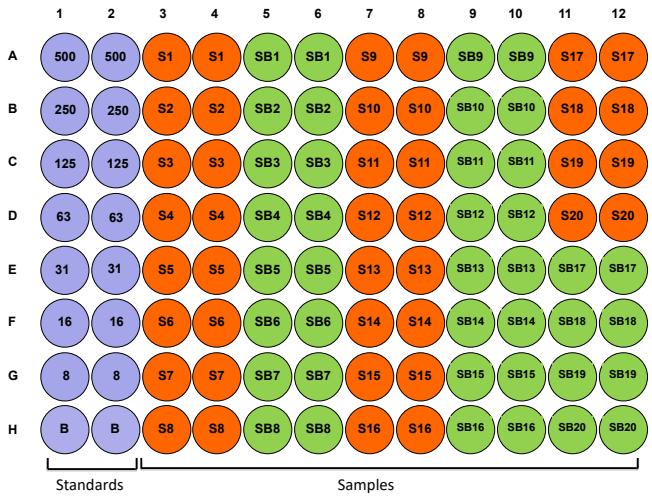
PLATE MAPPING

Map the plate based on the number of samples and sample dilution. For example, if sample dilution is higher than 1:200, it is not necessary to run antigen un-coated wells, but if sample dilution is less than 1:200, 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.

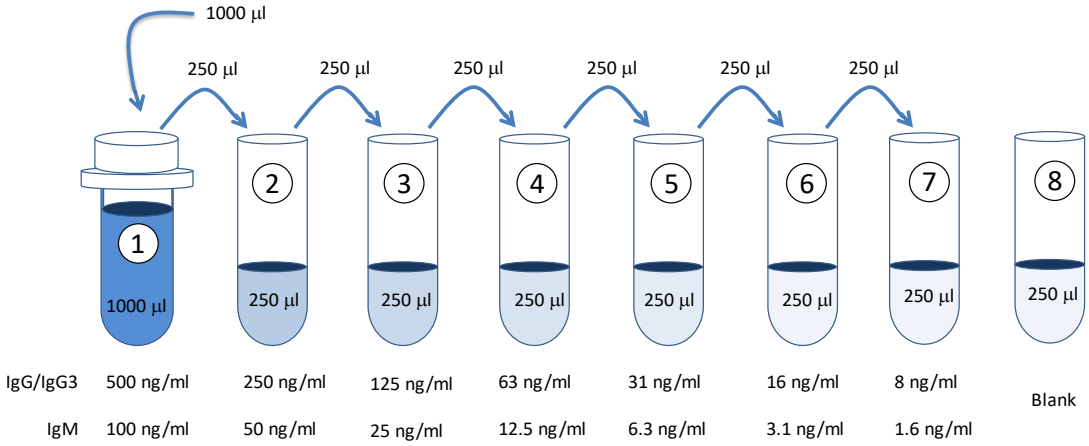
NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY 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 1:200 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" below. 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 1:200 due to low antibody levels, antigen uncoated control strips will be necessary. Please contact support@chondrex.com for more information.

   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 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 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 blotting 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 solution in 10 ml Chromogen Dilution Buffer. The prepared TMB cannot be stored for the next 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 blank, standards, and test samples.

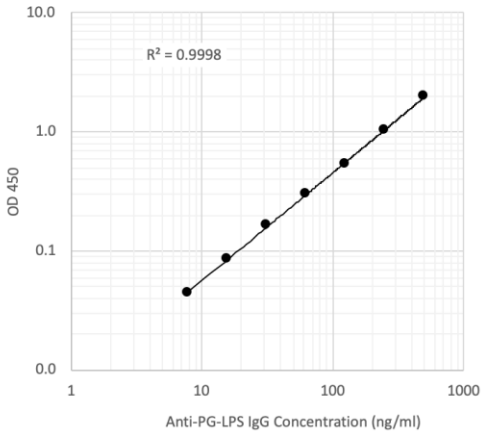
2. Subtract the "blank" (B) values from the averaged OD values in step 1.

   NOTE: If 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.

1. Plot the OD values of standards against the concentration of standard antibody (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 8 - 500 ng/ml.

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

Figure 1 - A Typical Standard Curve for the Mouse Anti-PG-LPS IgG Antibody ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility data for Mouse Anti-PG LPS IgG Antibody ELISA Kit

Test	16 ng/ml	63 ng/ml	250 ng/ml
Intra-Assay CV (%)	5.6	8.6	9.5
Inter-Assay CV (%)	4.6	1.8	5.2
Spike Test* (%)	105%	100%	98%

Table 2 - Reproducibility data for Mouse Anti-PG IgM Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	2.7	4.1	3.0
Inter-Assay CV (%)	2.9	3.8	9.5
Spike Test* (%)	90%	95%	97%

Table 3 - Reproducibility data for Mouse Anti-PG IgG3 Antibody ELISA Kit

Test	16 ng/ml	63 ng/ml	250 ng/ml
Intra-Assay CV (%)	3.5	7.0	3.9
Inter-Assay CV (%)	9.2	5.7	9.4
Spike Test* (%)	98%	103%	108%

* Known amounts of anti-PG LPS antibodies were added to standards and then diluted with Sample/Standard Dilution Buffer to assay anti-PG antibodies

TROUBLESHOOTING

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

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. J. Taurog, J. Richardson, J. Croft, W. Simmons, M. Zhou, *et al*., The Germfree State Prevents Development of Gut and Joint Inflammatory Disease in HLA-B27 Transgenic Rats. *J Exp Med* **180**, 2359-64 (1994).
15. Z. Reháková, J. Capková, R. Stěpánková, J. Sinkora, A. Louzecká, *et al*., Germ-free Mice Do Not Develop Ankylosing Enthesopathy, a Spontaneous Joint Disease. *Hum Immunol* **61**, 555-8 (2000).
16. P. Anderlik, I. Szeri, Z. Bános, Z. Barna, Bacterial Translocation After Cold Stress in Young and Old Mice. *Acta Microbiol Hung* **37**, 289-94 (1990).
17. A. Velin, A. Ericson, Y. Braaf, C. Wallon, J. Söderholm, Increased Antigen and Bacterial Uptake in Follicle Associated Epithelium Induced by Chronic Psychological Stress in Rats. *Gut* **53**, 494-500 (2004).
18. I. Khalif, E. Quigley, E. Konovitch, I. Maximova, Alterations in the Colonic Flora and Intestinal Permeability and Evidence of Immune Activation in Chronic Constipation. *Dig Liver Dis* **37**, 838-49 (2005).
19. J. Marchesan, E. Gerow, R. Schaff, A. Taut, S. Shin, *et al*., Porphyromonas Gingivalis Oral Infection Exacerbates the Development and Severity of Collagen-Induced Arthritis. *Arthritis Res Ther* **15**, R186 (2013).
20. K. Sato, N. Takahashi, T. Kato, Y. Matsuda, M. Yokoji, *et al*., Aggravation of Collagen-Induced Arthritis by Orally Administered Porphyromonas Gingivalis Through Modulation of the Gut Microbiota and Gut Immune System. *Sci Rep* **7**, 6955 (2017).
21. K. Terato, D. Harper, M. Griffiths, D. Hasty, X. Ye, *et al*., Collagen-induced Arthritis in Mice: Synergistic Effect of E. Coli Lipopolysaccharide Bypasses Epitope Specificity in the Induction of Arthritis With Monoclonal Antibodies to Type II Collagen. *Autoimmunity* **22**, 137-47 (1995).