Instruction Manual No. M-680-610-LHG

Human Anti-Lipopolysaccharide (Anti-LPS, E. coli 0157:H7, EHEC)) IgG

ELISA KIT # 680-610-LHG

For the detection of IgG antibody to LPS (E. coli)157:H7) in human serum or plasma.

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

Kit Components (96 tests)	
LPS antigens coated strip plate, (8x12 strip or 96 wells) # 680611	1 plate
Std A (0.65 mL; Human Anti-LPS (O157:H7) IgG 0 U/ml #680612A	1 vial
Std B (0.65 mL; Human Anti-LPS (O157:H7) IgG 6.25 U /ml #680612B	1 vial
Std C (0.65 mL; Human Anti-LPS (O157:H7) IgG 12.5.0 U /ml #680612C	1 vial
Std D (0.65 mL; Human Anti-LPS (O157:H7) IgG 25 U /ml #680612D	1 vial
Std E (0.65 mL; Human Anti-LPS (O157:H7) IgG 50 U /ml #680612E	1 vial
Std F (0.65 mL; Human Anti-LPS (O157:H7) IgG 100 U /ml #680612F	1 vial
All standards are calibrated to an internal reference using arbitrary units.	
Anti-Human IgG-HRP Conjugate,100X (0.15 ml) #H-HuG-612	1 bottle
Sample/Conjugate Diluent (20X), 10ml # SD-20T	1 bottle
Low NSB Sample Diluent (green color), 30 ml # Tbtm	1 bottle
Wash buffer (100X) 10 ml # WB-100	1 bottle
TMB Substrate Solution, 12 ml #80091	1 bottle
Stop Solution, 12 ml # 80101	1 bottle
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Intended Use

ADI LPS (E. coli O157-:H7, EHEC) IgG ELISA Kit is intended for the detection of IgG antibody to LPS (O157:H7) in human serum or plasma. This kit is for in vitro research use only.

Introduction

Escherichia coli O157:H7 is an enterohemorrhagic serotype of the bacterium Escherichia coli (EHEC) and a cause of illness, typically through consumption of contaminated food. Transmission is via the fecal-oral route, and most illness has been through distribution of contaminated leaf green vegetables. In some people, particularly children under five years of age and the elderly, the infection can cause hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail. About 2–7% of infections lead to this complication. In the United States, HUS is the principal cause of acute kidney failure in children, and most cases of HUS are caused by E. coli O157:H7. EHEC strains possess several virulence factors that include Shiga (Vero) cytotoxins and Lipopolysaccharides (LPS) that may act in synergy.

Lipopolysaccharides (LPS), also known as lipoglycans, are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals. LPS is the major component of the Bacterial cell wall of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. It is of crucial importance

to gram-negative bacteria, whose death results if it is mutated or removed. LPS acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex, which promotes the secretion of pro-inflammatory cytokines in many cell types, but especially in macrophages and B cells.

LPS contains three parts: O antigen (or O polysaccharide), Core oligosaccharide, and Lipid A. O antigen is repetitive glycan polymer contained within an LPS is referred to as the O antigen, O polysaccharide, or O side-chain of the bacteria. The O antigen is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. The presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. O antigen is exposed on the very outer surface of the bacterial cell, and, as a consequence, is a target for recognition by host antibodies. **Core oligosaccharide** always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO, keto-deoxyoctulosonate). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substitutents. **Lipid A** in normal circumstances, a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock). The Lipid A moiety is a very conserved component of the LPS. Bacteria with common serotypes have surface antigens (group O, group H, or LPS) which generate the same antibody response. Examples of serotypes are O55:B5 and O26:B6 for the E. coli bacterium. The designations are immunological classifications, which specify which antibody recognized which strains. Different strains may have some common antigenic determinants.

Humans are much more sensitive to LPS than other animals (e.g., mice). A dose of 1 µg/kg induces shock in humans, but mice will tolerate a dose up to a thousand times higher. This may relate to differences in the level of circulating natural antibodies between the two species. While the significance of natural anti-LPS antibodies is not entirely clear, one proposed role is clearance of bacterial LPS. Gram-negative bacterial sepsis remains a major cause of lethality in hospitalized patients, despite routine therapy. Both polyclonal (anti-LPS IgG/IgM) and monoclonal antibody preparations directed against the common deep core/lipid A region of LPS are cross-reactive in vitro and cross-protective in vivo against a wide range of challenge organisms and LPS, and preliminary clinical trials indicate that a reduction in lethality may be possible. Therefore, higher basal level of anti-LPS IgG or IgM may offer better protection against bacterial infection or septicemia. The demonstration of anti-LPS antibodies in clinical samples is of diagnostic value in certain Gram-negative bacterial infections.

PRINCIPLE OF THE TEST

ADI's LPS antibody IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA or ELISA). Diluted serum or plasma samples are added to wells coated with purified LPS antigens. LPS-specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme that produced blue color. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

All human derived material has been tested negative for HIV, HCV, and HbSAg. Nevertheless, all precautions should be taken and samples be treated as potentially hazardous.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:100 or higher. We recommend making an initial stock of 1:10 in 1X-sample diluent. The antibodies are stable in this buffer and kept at 4oC for up to 2-4 weeks. Make additional test dilution of samples from 1:10 stock (e.g., for 1:500 dilution, dilute 1:10 stock by another 1:50 or 10-ul of 1:10 stock and 490 ul of Low Nsb diluent . In this way, sample can be tested from the same stock and avoid freez and thaw.

We recommend human sample testing at 1:500 dilution of mouse sample in Low NSb diluent (green). This buffer keeps the non-specific binding and the final A450 <0.300. If samples are diluted 1:500 in 1X sample/conjugate diluent then the non-specific A450 of normal human samples may be higher (0.5-0.8).

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:100 with water. (**Dilute 10 ml stock with 990 ml distilled water**) Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.
2. **Prepare 1X Sample/Conjugate Diluent**. Prepare 1X working stock by dilution 1:20 with water. Dilute 1 ml stock in 19 ml water. This diluent can be used to prepare initial sample stock of 1:10 and dilute the antibody-HRP conjugate.
3. **Prepare 1X anti-human IgG-HRP conjugate**. Stock is provided as 100X stock. Dilute 10 ul stock conjugate in 1 ml of 1X sample/conjugate diluent prepare above. Prepare 12 ml working stock for each strip or 12 ml for full plate assay (120 ul stock conjugate in 12 ml of 1X conjugate diluent)..

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{O}C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:100 or higher with the sample diluent (see page 3 for recommended preparation of the sample dilution). It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. DO NOT dilute calibrators or controls. **Dilute wash buffer stock (100X) 1:100 with distilled water. Prepare 1X-HRP conjugate by diluting 1:100 with 1X conjugate diluent.**

1. Label or mark the microtiter well strips to be used on the plate

2. Dispense **100 ul** 1X sample diluent in duplicate wells to be used as blank. Pipet **100 ul of** calibrators, controls, and diluted samples into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds and incubate at room temp (25-28oC) **for 60 min**.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul anti-human IgG-HRP conjugate** to all well. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (18-26oC).

5. Wash the wells 4 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. **Blue color** develops in positive controls and samples.

7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

8. Measure the **absorbance at 450 nm** using an ELISA reader within 60 min.

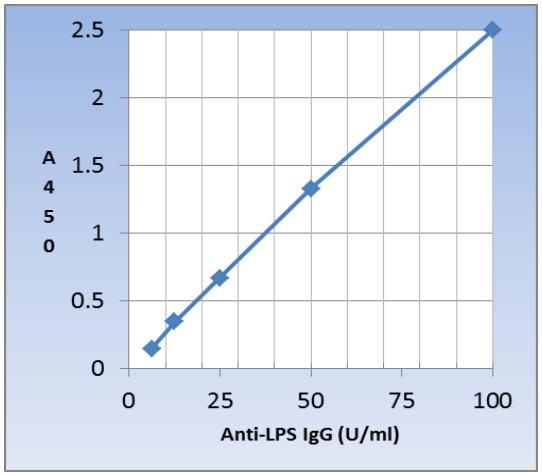
NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Do not touch the bottom of the wells.

WORKSHEET OF A TYPICAL ASSAY

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

Serum	U/ml	A450	Net A450 (minus blank)
Sample Diluent or Blanks	0.00	0.100	
Human Anti-LPS IgG Std A	6.25	0.250	0.15
Human Anti-LPS IgG Std	12.5	0.435	0.335
Human Anti-LPS IgG Std C	25	0.77	0.67
Human Anti-LPS IgG Std D	50	1.43	1.33
Human Anti-LPS IgG Std E	100	2.60	2.50



Typical Std Curve (do not use this for sample calculation)

CALCULATION OF RESULTS

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis,) either on lin-lin graph paper or point-to-point curve fit. Results of unknown samples have to be multiplied by the dilution factor (e.g., 1:500 have to be adjusted for the dilution factor and multiplied by 500).

Unknowns showing concentrations above the highest calibrator have to be diluted as described in “Assay Procedure” and re-assayed.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
The A450. of the blanks should be <0.50 and the highest standard >1.00. High blank values >0.300 are usually due to inefficient washing, particularly after the antibody-HRP conjugate step. Increase the # of washing and make sure to tap the plates over a paper towels after the final wash or in between washes if washing manually

Normal Human Sample Testing

A random sampling or human serum samples were tested in anti-LPS IgG and anti-LPS IgM ELISA at dilution of 1:500. In general, there is higher prevalence of IgG than IgM. A similar analysis of mouse sample showed negligible anti-LPS IgG/IgM. So there appears to be species differences as well as antibody isotype against LPS. Note: **Samples*** with asterisk were above the linear range of reading and showed high antibody levels at 1:5000, and 1:10,000. So we recommend testing of high IgG samples at several dilutions to assess accurate sample values.

#	Anti-LPS IgG A450	Anti-LPS IgM A450
1.	0.693	0.056
2.	0.409	0.248
3.	1.385	0.272
4.	0.569	0.170
5.	0.384	0.041
6.	0.246	0.184
7.	0.272	0.193
8.	1.050	0.295
9.	0.211	0.085
10.	0.645	0.033
11.	0.371	0.055
12.	0.479	0.230
13.	0.350	0.051
14.	0.163	0.158
15.	0.391	0.168

Cut-Off Values

In our testing, if human serum samples are diluted at 1:500, a basal reading of A450=0.2-0.4 even in sample that were neutralized with purified LPS. So this value may be considered as cut-off (basal or non-specific) values for Anti-LPS IgG. All samples with 0.600 (an arbitrary) cut-off values may be considered positive. However, we recommend that the users establish their own cut-off values and normal ranges for a given sample pool.

References: Nvarro A (2003) Clin Diagn Lab Immunol.10(5):797-801; Chart H (2002) J Med Microbiol. 51(6):522-5; Currie CG (2001) J Med Microbiol. 50(4):345-54; Laegreid W (1998) Clin Diagn Lab Immunol. 5(2):242-6; Westerman RB (1997) J Clin Microbiol. 35(3):679-84; tsutsumi R (2004) Microbiol Immunol. 48(1):27-38; Palmeria P (2007) Eur J Pediatr. 166(5):413-9

Specificity

Highly purified LPS from E. coli K-235 was used in the kit is obtained and purified by gel filtration methods. LPS contains three parts: O antigen (or O polysaccharide), Core oligosaccharide, and Lipid A. Most antibodies are made to lipid A moiety but this kit will not distinguish the antibodies made to the O antigen or Lipid A. Anti-human IgG-HRP conjugate has been optimized to detect all IgG subtypes (IgG1-4) but not the IgM or IgA.

References: Thirumalapura NR (2005) J. Imunol. Methods. 298, 73-81; Dunn Dl (1990) J. Trauma S100-106; Young LS (1989) Re. Infec. Dis. Suppl. 7, S1564-71; Fink MP (1993) 2 Suppl S32-9; Chandra T (1991) Prog. Clin. Res. 367, 141-159; Horner C (2004) Anaesthediol. 53, 10-28; Jackson Sk (1993) 9, 540-545; Pluschke G (1985) Infec. Immun. 49, 365-370.

ELISA kits available from ADI (see details at the web site)

Catalog#	ProdDescription
4200	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit
4205	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit
520-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests
970-100-PHG	Human Anti-Poliomyelitis Viruses 1-3 IgG ELISA Kit, 96 tests
970-120-PMG	Mouse Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
970-150-PMG	Monkey Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
970-160-VPG	Mouse Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) IgG ELISA
970-170-VPG	Human Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) IgG ELISA
600-020-HRV	Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-120-HRV	Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 2x 96 tests,
600-220-HRV	Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 2x 96 tests,
600-300-115	Human Anti-Meningococcal Group ACWY Oligosaccharides-Diphtheria CRM197
700-140-KLM	Human Anti-KLH IgG (total) ELISA Kit, 2x 96 tests, Quantitative
700-160-VAH	Human Anti-Vacmune/Immucothel (KLH) IgG (total) ELISA Kit,2x 96 tests,
900-160-83T	Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
910-160-JEM	Human Anti-Japanese encephalitis virus (JEV) IgG specific ELISA kit
920-040-HAG	Human Anti-Influenza A virus IgG ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgM ELISA kit
920-400-HBG	Human Anti-Influenza B virus Ig's ELISA kit
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-100-DHG	Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit
950-110-AHG	Human Anti-Adenovirus IgG ELISA kit
950-120-AHM	Human Anti-Adenovirus IgM ELISA kit
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit
980-100-PHG	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit, 96
980-110-PHM	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit, 96
990-100-THA	Human Anti-Mycobacterium Tuberculosis IgA ELISA kit, 96 tests
990-110-THG	Human Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests
990-120-THM	Human Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests
AE-320420-1	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96 tests
AE-320430-1	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96 tests
AE-320520-1	Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests