INTENDED USE

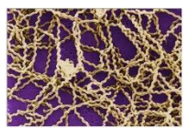
The Human Anti-Leptospira LipL32 (Anti-LipL32) ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity specific for Leptospira in serum, plasma, or other qualified biological samples from vaccinated, immunized, and/or infected hosts.

This immunoassay is suitable for:

- Determining immune status relative to non-immune controls
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization, and timing
- Qualifying and/or standardizing vaccine batches and protocols

This kit is for research use only (RUO), not for diagnosis or therapeutic purposes.

INTRODUCTION

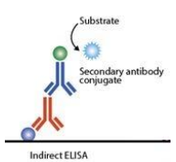


Leptospirosis is an infectious disease of humans and animals, mainly dogs, cattle, horse, and swine. It is caused by pathogenic spirochetes, a flexible twisted bacterium, of the genus Leptospira. It is considered the most common zoonosis in the world. Humans acquire leptospirosis either from direct contact with the urine of infected animals, or from contact with material contaminated by it, such as water or soil. In domestic animals, the leptospiral infection causes poor reproductive performance, abortions, and premature births, which lead to economic losses.

Pathogenic *Leptospira* species are capable of surviving as free-living bacteria for extended periods of time after urinary shedding. Leptospiral infection can be detected by microagglutination tests (MAT). This tests is laborious and less sensitive. Recently, the Leptospiral antigenic protein LipL32, has been used to detect antibodies to Leptospira by ELISA. Seroconversion or increasing antibody titers in paired serum specimens provide strong evidence for true infection, but the samples need to be taken 2 to 3 weeks apart.

Leptospirosis vaccines: A number of vaccines are available for animal use: Lepto EQ Innovator (Leptospira Pomona) Equine Vaccine. Nobivac Lepto 4 is recommended as an aid in the prevention of disease, urinary shedding, and mortality caused by lepto strains L. canicola, L. icterohaemorrhagiae, L. pomona, or L. grippotyphosa.

PRINCIPLE OF THE TEST



The **Human Anti-LipL32 IgG** ELISA kit is based on the binding of human anti-LipL32 IgG in samples to LipL32 immobilized on the microwells. Anti-LipL32 IgG antibody is detected by anti-Human IgG specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-LipL32 IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgG antibody in samples is calculated relative to anti-LipL32 calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8° C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4° C for long term and RT for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8° C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
LipL32 Coated Strip Plate	500601	8-well strips (12)	Coated with LipL32 proteins; post-coated with stabilizers.
Anti- LipL32 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	500602B 500602C 500602D 500602E	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti- LipL32 antibodies; in buffer with antimicrobial.
Anti- LipL32 Positive Control	500602 PC	0.65ml	Anti- LipL32 diluted in buffer with protein, detergents, and antimicrobial. [Value range on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution
Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same week as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/100): **10**ul initial (1/5) + 1**90**ul LNSD (1/100)

Assay Design
Review Interpretation of Results (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the Anti- **LipL32 Positive Control**; the value range is on the label.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

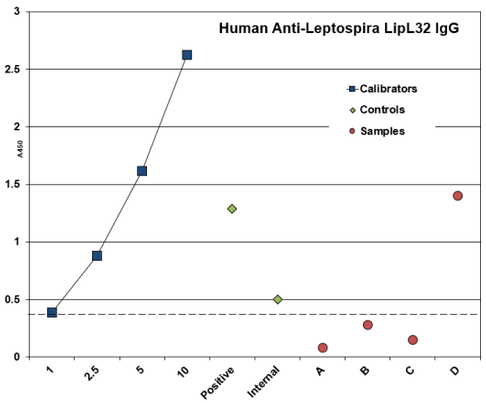
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti- LipL32 IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from LipL32 protein immunization shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – serum showing reactivity to the LipL32 protein; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:50) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

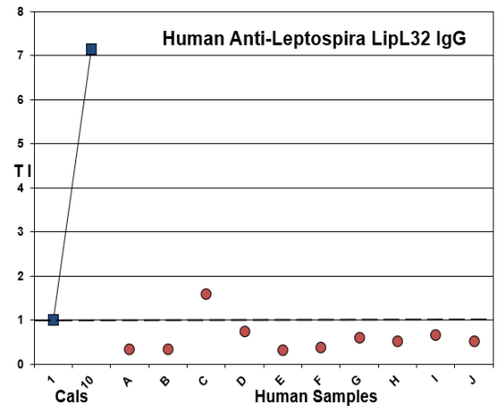
- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum IgG

A panel of sera/plasma of unknown history was tested for anti-LipL32 IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Calibrator**.



Results

Anti-LipL32 IgG:

Nine of the human sera/plasma were negative (clearly below 1.0 TI) and one (C) was positive at 1:100 dilution.

Notes:

1. **Positives** may be due to prior encounter with the bacteria, from exposure to an antigen with common epitopes, or from immunization.
2. When the **Positive Index** is **above 5.0,** using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

Specificity

The antigen is recombinant Leptospirosis lipoprotein LipL32 expressed in *E.coli* as a His-tag fusion protein (261 aa, >95% purity, ~35 kDa) derived from *Leptospira interrogans* serogroup Icterohaemorrhagiae, serovar Lai, strain Fiocruz L1-130.

LipL32 is highly conserved among pathogenic *Leptospira* species; the protein shows 98–100% amino acid sequence identity with LipL32 from *Leptospira santarosai*, *Leptospira noguchii* serovar Pomona, *Leptospira noguchii* serovar Valbuzzi, *Leptospira borgpetersenii* serovar Australis, *Leptospira kirschneri* serovar Bim, *Leptospira kirschneri* serovar Zanon, *Leptospira kirschneri* serovar Paidjan, *Leptospira kirschneri* serovar Panama, *Leptospira borgpetersenii* serovar Hardjo-bovis, and numerous other pathogenic serovars. Lower sequence identity (approximately 70%) is observed with LipL32 from *Leptospira broomi* serovar Hurstbridge and *Leptospira inadai* serovar Lyme, among others.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

Instruction Manual No. M-RB-500640-1

Human Anti-Leptospira LipL32 IgG ELISA Kit

Cat. #RB-500640-1, 96 tests

For Quantitation of Anti-LipL32 IgG in Serum, Plasma, or other Biological Fluids

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	Amount	Part #
LipL32 Coated Strip Plate	8-well strips (12)	500601
Anti- LipL32 Positive Control	0.65 ml	500602PC
Anti- LipL32 Calibrator 1 U/ml	0.65 ml	500602B
Anti- LipL32 Calibrator 2.5 U/ml	0.65 ml	500602C
Anti- LipL32 Calibrator 5 U/ml	0.65 ml	500602D
Anti- LipL32 Calibrator 10 U/ml	0.65 ml	500602E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-RB-500640-1