INTENDED USE

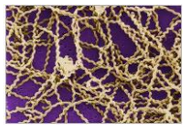
Human Anti-Leptospira lipoprotein LipL32 **(Anti-LipL32 IgG)** ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity specific for Leptospira in serum, plasma or other qualified biological samples from vaccinated, immunized and/or infected hosts.

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 standardizing vaccine batches & protocols.

The assay is for research use only (RUO) and is not intended nor validated for therapeutic or diagnostic uses.

GENERAL INFORMATION

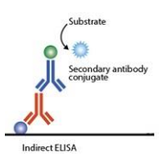


Leptospirosis is an infectious disease of humans and animals mainly dogs, cattle, horse, and swine. It is caused by pathogenic spirochetes, a flexible twisted bacterium, of the genus Leptospira. It is considered the most common zoonosis in the world. Humans acquire leptospirosis either from direct contact 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, 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, 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 Anti-LipL32 Ig's (IgA/IgG/IgM) ELISA kits are based on the binding of antibodies in samples to the purified LipL32 antigen immobilized on the microwells. Bound antibody is detected by anti-IgG or IgM-HRP conjugate (species specific). After a washing step, chromogenic substrate (TMB) is added and color (blue) developed, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added to terminate the reaction, and Absorbance (yellow color) is then measured using an ELISA reader at 450nm. The presence of antibody (IgA/IgG/IgM) in samples is determined relative to anti-LipL32 Ig's Calibrators and Controls.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}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 (50x) Cat. #WB-50, 10 ml	Dilute the entire volume 10ml + 490 ml with distilled or deionized water into a clean stock bottle. Label as ***1X* Wash Solution** and store at 4°C for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20TG, 10 ml (green solution)	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as 1**X Working Sample Diluent** (WSD) and store at 2-8°C until the kit lot expires or is used up.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
LipL32 Coated Strip Plate	500641	8-well strips (12)	Coated with LipL32 and post-coated with stabilizers.
Anti-LipL32 IgG Calibrators			
3 U/ml **10 U/ml** **30 U/ml** **100 U/ml**	500642A 500642B 500642C 500642D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-LipL32 in buffer with antimicrobial.
Anti-LipL32 IgG positive Control	500643 -PC	0.65 ml (red cap)	Human serum with anti- LipL32 IgG reactivity; **Net OD > 0.6**
Low NSB Sample Diluent (LNSB)	TBTm **Not** for HRP dilution	30 ml	Buffer with protein, detergents and anti-microbial. Use as is for sample dilution to suppress non-specific binding.
Anti-Human IgG- HRP Conjugate	RCH-1-1	150 ul (100X)	provide in buffer with detergents and antimicrobial. Dilute 1:100 with 1XWSD
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 for diluting samples and Anti-Camel IgG HRP Concentrate.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength 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.

Sample Dilution & Antibody Stability

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 5-10 times the initial dilution and performed the same day as the assay.

Example: Initial (1/10): **10**ul serum + **90**ul of 1X WSD
Further (1/20):**10**ul initial (1/10) + 18**0**ul LNSD (final dilution 1/200)
Other dilution such as 1:500 should be made from the 1:10 stock.

Assay Design

Review Interpretation of Results (p5-7) 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 **3 U/ml Calibrator**. This is usually 1:200 or greater dilution for serum with normal levels of IgG.
- Run the Anti-LipL32 IgG Positive Control; net OD > **0.5**.
- 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 a set of **Calibrators**, which validate that the assay was performed to specifications: **100 U/ml** should give a high signal (>1.0 OD); **3 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 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 Antibody-HRP to each well.
- Tap the plate gently to mix reagents, Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Tap the plate gently to mix, 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

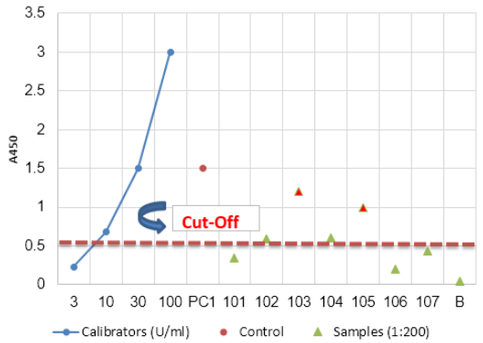
- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If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

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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Net A450
A1/2	Blanks (sample diluent)	0.100	-
B1/2	Calibrator A (3 u/ml)	0.55	0.43
C1/2	Calibrator B (10 u/ml)	0.873	0.773
D1/2	Calibrator C (30 u/ml)	1.215	1.15
E1/2	Calibrator D (100 u/ml)	2.88	2.78
F1/2	**Positive Control**	1.32	1.22
G1/G2	**S1@1:200**	0.45	0.35
H1/H2	**S2 @1:200**	0.98	0.88



Anti-LipL32 IgG in Humans

Soum/3_Soum-ELISA

The above graphs is for demonstration purpose only. Actual values may differ lot to lot but be close to the above illustration. Use the lot specific curve for calculating the sample values.

INTERPRETATION OF RESULTS

Qualitative Results

1. Calculate the average A450 of the blanks, -ve and positive control.
2. Subtract the average blanks values from the average values of the controls and samples.
3. **Arbitrary Cut-off values**: Add 0.10 to the negative control values of negative control values supplied with the kit or User supplied –ve control (if available). A redline has been drawn in the above graphs to represent the '**Cut-off values**"
4. **Sample values at or below the cut**-off values can be treated as –ve and above it are positive.

Examples: Net Average negative control values =0.405
*__Cut-Off__: Add 0.100 (0.405+0.100) =0.505
Negative samples <0.505
Positive samples >0.505

**Arbitrary cut-off is based upon our regional sample analyses. We strongly recommend that users set-up their own negative and cut-off values based upon samples from the region. No single cut-off values may truly represent samples from all over the world.

Quantitative Results

1. Calculate the average A450 of the blanks, calibrators controls, and samples
2. Subtract the average blanks values from the average values of the calibrator and samples
3. Plot the net average A450 values of calibrators against the concentration (u/ml).
4. We recommend using point-to-point graphs.
5. Calculate the unknown sample values from the graph.
6. Multiply the values by the dilution factor of the samples.

The **sensitivity** of the assay to detect anti-MERS-NP 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 camel serum diluted 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-MERS-NP antibody, derived from MERS vaccination, 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.

INTERPRETATION OF RESULTS

1. **Positives** may be due to prior encounter with the virus or vaccination immunization.

2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution >1:200 (e.g., 1/500) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1/100) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative "Cut-off" should be considered using known negatives (Page 5).

Alternative Methods. 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 Positive and Sensitivity Control values can be used to normalize inter-assay values.

Calculations

On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive

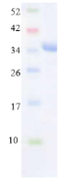
sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

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.
= IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

Antigen Specificity



Recombinant Leptospirosis lipoprotein LipL32 was expressed in E.coli as a His-tag fusion protein (261 aa, >95%, ~35 kda; Serogroup Icterohaemorrhagiae/Serovar lai/ Leptospira interrogans (strain Fiocruz L1-130)). is used as antigen. The protein is 98-100% conserved in various serotypes: (strain Fiocruz L1-130), Leptospira santarosai, Leptospira noguchii serovar Pomona, serovar Valbuzzi (100%), Leptospira borgpetersenii; serovar Australis, kirschneri serovar Bim, serovar Zanon, serovar Paidjan, serovar Panama and many others. serovar Hardjo-bovis. Appriximately 70% in Leptospira broomii serovar Hurstbridge; Leptospira inadai serovar Lyme and many others.

Leptospirosis in Humans

Although many wild and domestic animals can serve as reservoir hosts, the brown rat (Rattus norvegicus) is the most important source of human infections. Individuals living in urban slum environments characterized by inadequate sanitation and poor housing are at high risk of rat exposure and leptospirosis. Antibody titers must be established on a paired samples taken 2-3 weeks to apart to establish an increase in antibody titers. A fourfold or greater rise in titre between paired sera confirms the diagnosis, regardless of the interval between samples. Regardless, any diagnosis of the infection must be established 2 or more different tests such as ELISA, PCR, or IHVC.

Human Vaccines: This kit can be used to monitor the increase in antibody titer to LipL32 upon vaccination. A whole-cell based, killed vaccines have been tested in humans.

References: Haake DA (2015) Curr Top Microbiol Immunol. 2015; 387: 65–97; Goris MGA (2013) PLOS Negl. Trop. Dis. 7, e2290; Mailloux M (1983) Med. Hyg. 41, 1025-1030; Martinez R (2004) Rev Panam Salud Publica. 2004;15:249–255

Catalog#	Description
RB-500600-1	RecomBac Pig/Swine Anti-Leptospira LipL32 IgG ELISA
RB-500610-1	RecomBac Bovine Anti-Leptospira LipL32 IgG ELISA kit
RB-500620-1	RecomBac Goat/Sheep Anti-Leptospira LipL32 IgG ELISA kit
RB-500630-1	RecomBac Mouse Anti-Leptospira LipL32 IgG ELISA kit
RB-500640-1	RecomBac Human Anti-Leptospira LipL32 IgG ELISA kit,
RB-500650-1	RecomBac Dog Anti-Leptospira LipL32 IgG ELISA kit

Instruction Manual No. RV-402110

RecomBac Human Anti-Leptospira LipL32 protein IgG ELISA kit

Cat # RB-500640-1, 96 tests

For Quantitation of Anti-LipL32 IgG in Serum or Plasma or other biological fluids

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

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Draft version-Please consult the manual supplied with the kit for any lot specific change.