Human Leptospira antibody (IgG) ELISA Kit

Cat#: orb406334 (ELISA Manual)

PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique.
The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells and any antibodies specific for the antigen present will bind to the pre-coated antigen. After removal of unbound material, alkaline phosphatase-conjugated anti-human IgG from goat is added to each well and react with the immune complex. Finally, substrate solution ρ-nitrophenylphosphate is added to each well. The enzyme-substrate reaction is terminated by the addition of sodium hydroxide and the color change is measured spectrophotometrically at a wavelength of 405 nm ± 2 nm. Calculate the concentration of human Leptospira antibody (IgG) in the samples.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1(96 wells)
Negative Control	1x 2 ml
Standard Serum	2 x 2 ml

Conjugate	1 x 13 ml
Wash Buffer(30 x concentrate)	1 x 33.3 ml
Sample Diluent	2 x 50 ml
Substrate	1 x 13 ml
Stop Solution	1 x 15 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

Unopened kit		Store at 2 -8°C. Do not use the kit beyond the expiration date.
Opened kit	Assay plate	May be stored for up to 1 month at 2 -8°C. Try to keep it in a sealed aluminum foil bag and avoid the damp.
Negative Control		May be stored until expiry date at 2 -8°C.
Standard serum		May be stored until expiry date at 2 -8°C.
Conjugate		May be stored until expiry date at 2 -8°C.
Wash Buffer		Working dilution may be stored for up to 2 weeks at 2 -8°C, or 1 week at room temperature.
Sample Diluent		May be stored until expiry date at 2 -8°C.
Substrate		May be stored until expiry date at 2 -8°C.
Stop Solution		May be stored until expiry date at room temperature.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm, reference wavelength between 620 nm and 690 nm (e.g. 650 nm).
- An incubator which can provide stable incubation conditions up to37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500mlgraduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

The Stop Solution provided with this kit is an aqueous slkali. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g, 2 -8°Cwithin 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

SAMPLE PREPARATION

Samples require a 101-fold dilution into Sample Diluent before test. The suggested 101-fold dilution can be achieved by adding 10µl sample to 1000µl of Sample Diluent.

Note:

1. Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Samples must not be thermally inactivated.
4. Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
5. Obviously contaminated samples (serum or plasma) should not be tested due to the risk of wrong results.
6. Grossly hemolyzed samples are not suitable for use in this assay.
7. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
8. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

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Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 33.3 ml of Wash Buffer Concentrate (30 x) into deionized or distilled water to prepare 1000 ml of Wash Buffer (1 x).

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and controls be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Substrate blank** well (without any other solution).
4. Add 100µl of **Negative Control**, **Standard Serum** or **diluted sample** per well. Cover with the adhesive strip provided. Incubate for 60 minutes at 37°C. A plate layout is provided to record controls and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of **four** washes. Wash by filling each well with **Wash Buffer** (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer and let it stand for 20-40 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100µl of **Conjugate** to each well except Substrate blank well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at 37°C.
7. Repeat the aspiration/wash process for four times as in step 5.
8. Add 100µl of **Substrate** to each well (including Substrate blank well). Incubate for 30 minutes at 37°C. **Protect from light.**
9. Add 100µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Read OD within 60 minutes at 405 nm against Substrate blank. Reference wavelength between 620 nm and 690 nm (e.g. 650 nm).

*__Samples may require dilution. Please refer to Sample Preparation section.__

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrate (e.g. observation once every 10 minutes). If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrate in unopened bottle may have a slightly yellow coloring. This does not reduce the quality of the product!
7. Substrate is easily contaminated. Discard when solution turns quite yellow. Please protect it from light.

CALCULATION OF RESULTS

The Blank must be subtracted from all OD values prior to the evaluation.

1. Criteria of Validity

- Substrate Blank OD value must be <0.25.
- Negative Control must produce a negative test result.
- The validity range of this kit is 0.47-1.58
- The mean OD-value (after subtraction of the substrate blank!) of the Standard Serum must be within the validity range.
- The variation of OD-values may not be higher than 20 %.

If these criteria are not met, the test is not valid and must be repeated.

2. Calculation

Qualitative Evaluation

To fix the cut-off ranges multiply the mean value of the measured standard OD as follows:
OD = 1.117x MW(STD) with upper cut-off
OD = 0.895x MW(STD) with lower cut-off
Standard reference values OD =0.93
For example: If the measured mean absorbance value of the standard serum is 1.0 OD, the range of the cut-off is in between 0.895-1.117OD.

- While ODsample ≥ upper cut-off: Positive
- While lower cut-off ≤ ODsample ＜upper cut-off: Suspicious samples
- While ODsample ＜ lower cut-off: Negative

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