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Porcine Leptospira antibody (Leptospira-Ab) ELISA Kit

96Tests

Catalogue Number: NSL1512Po

Store all reagents at 2-8℃

Validity Period: six months

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Porcine Leptospira antibody (Leptospira-Ab) ELISA Kit

FOR RESEARCH USE ONLY

Drug Names

Generic Name: **Porcine Leptospira antibody (Leptospira-Ab) ELISA Kit**

Purpose

Our Porcine Leptospira antibody (Leptospira-Ab) ELISA Kit is to for the qualitative determination of Leptospira-Ab in Porcine serum, plasma, culture media or any biological fluid.

Principle

The ELISA is based on the the qualitative enzyme immunoassay technique. The Microplate provided in this kit has been pre-coated with an antigen specific to Leptospira-Ab, make it to solid-phase antigen. Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for Leptospira-Ab is added to each Microplate well and incubated, so the antigen-antibody-Enzyme labeled antigen complex is formed. Following a wash to remove any unbound reagent, then the TMB substrate solution is added to each well. Only those wells that contain Leptospira-Ab and HRP conjugated Leptospira antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of Leptospira-Ab is determined by comparing with the CUTOFF value.

Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microplate	1	2-8℃
5	Negative control	0.5ml×1 bottle	2-8℃
6	Positive control	0.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.

2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

Procedure

1. In the Microplate, number the corresponding micropores of the sample in sequence,leave two wells as negative control, two wells as positive control and one empty well as blank control. (blank control hole don't add samples and HRP-Conjugate reagent, the rest step operation are same)

2. Adding samples: Adding 50ul of Negative and positive control to the negative and positive control wells respectively. For sample wells, 40μl Sample dilution buffer and 10μl sample are added in it. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T).

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Determine the result

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Porcine Leptospira-Ab negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Porcine Leptospira-Ab positive.

Notes

1. Store the kit at 4° C upon receipt. The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Leptospira antigen-Coated plate, reseal them in zip-lock foil and keep at 4° C.

2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve

all the precipitates, which will not affect the results.

3. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
4. Please keep Substrate away from light.
5. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microplate Reader.
6. All the samples, washing buffer and wastes should be treated as infectious agents.
7. Reagents from different lots should not be mixed.

Storage and validity

1. Storage: 2-8℃.
2. Duration: 6 months