Version 17-04/24

User's Manual

Leptospira Hardjo IgG ELISA Kit

REF **DEIA2332BG**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Leptospira hardjo (LH) IgG ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG antibodies to Leptospira hardjo in bovine milk and serum.

General Description

Leptospira serovar hardjo is endemic in cattle populations, which causes economic losses in the dairy industry as a consequence of agalactia, abortion, stillbirth, birth of weak calves and reduced fertility. The hardjo infection is also an occupational zoonosis for those who work with cattle. Control schemes are being looked at in a number of countries, however, current tests are unsafe, insensitive, non-specific and complicated.

Creative-Diagnostics has developed a highly specific and sensitive ELISA test for the detection of IgG antibodies to both Leptospira borgpetersenii serovar hardjo bovis (HB) & Leptospira interrogans serovar hardjo prajitno (HP) in either sera or milk (bulk tank or from individual animals). The hardjo IgG test kit provides a means for the qualitative detection of IgG antibodies directed to LH in bovine serum/milk. The test may aid in the determination of the animal's serological status, or may aid in the diagnosis of disease associated with serovar hardjo.

Principles of Testing

The test system for the detection of IgG class antibodies against LH in cattle is designed in the format of an indirect ELISA. Antibodies against LH which are present in the bovine specimen will bind onto the wells of a microtitre plate (strip) supplied pre-coated with LH antigen. The bound antibodies are detected by using horseradish peroxidase (HRP) conjugated monoclonal anti-bovine IgG, which in turn are visualised by adding TMB solution. Any colored product is measured at 450 nm after adding stop solution. The color intensity of the solution depends on the antibody concentration in the test sample.

Reagents And Materials Provided

1. Pre-Coated Microtitre Strips 12 × 8 wells
2. Assay Diluent 1 × 15 ml
3. Positive Control 1 × 1.0 ml
4. Negative Serum Control 1 × 1.5 ml
5. Negative Milk Control 1 × 1.5 ml
6. Wash Buffer (30× Concentrate) 1 × 30 ml
7. HRP Conjugate 1 × 12 ml
8. TMB Solution 1 × 12 ml
9. Stop Solution 1 × 12 ml
10. Microplate Sealer 1 piece
11. Package Insert 1 copy

*To avoid erroneous results, only use reagents and components from this kit and not from another batch.

Materials Required But Not Supplied

1. Microtitre plate reader equipped with 450 nm and 620-630 nm filters
2. Single and multichannel micropipettes from 50 µl to 1.0 ml, with disposable pipette tips
3. Microtubes (e.g. Eppendorf)
4. Microtiter plate washer/aspirator
5. Assorted glassware for the preparation of reagents and buffer solutions
6. Plate shaking equipment
7. Vortex mixer
8. Timer
9. Incubator
10. Distilled or deionised water
11. Absorbent pads (tissue)

Storage

The kit will remain stable until the expiration date, provided it is stored between 2-8°C. Unused strips and wells should be sealed in the foil pouch and stored as previously described. Do not freeze.

Specimen Collection And Preparation

Serum or milk may be used. If the specimen is to be tested within 7 days, it may be stored at +2 to +8°C. Otherwise specimens must be stored at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

DILUTION OF SPECIMENS

1. Dilute each serum specimen by 1:30 in a microtube using assay diluent (e.g. 8 µl of specimen added into 232 µl of assay diluent). Make sure to gently mix the serum specimens before taking them from their containers.
2. Milk specimens can be added directly to the well neat and undiluted.

Reagent Preparation

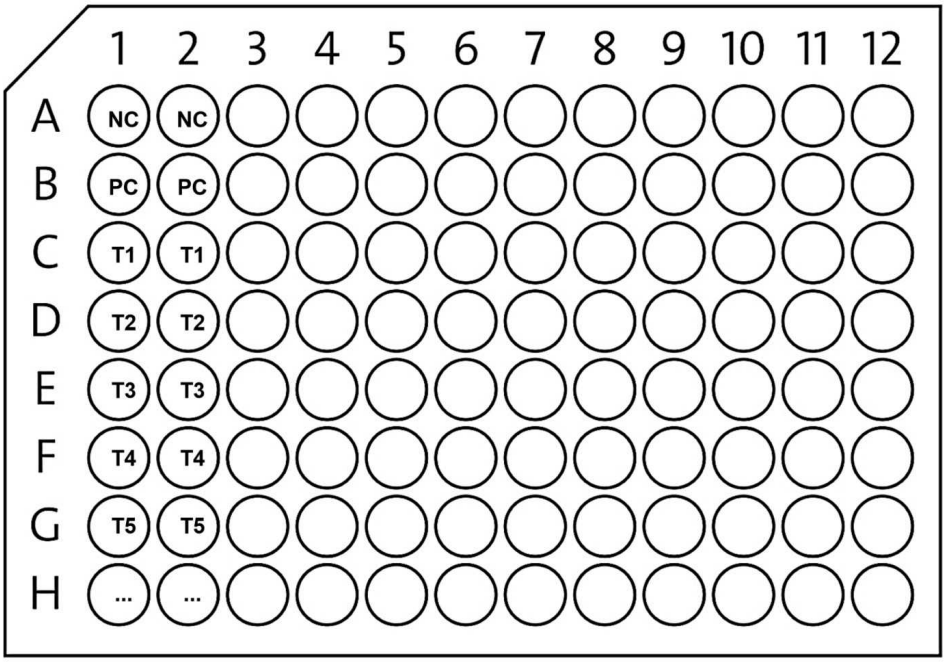
Wash buffer: Dilute the wash buffer concentrate (30×) 30-fold with ddH_2O (e.g. 10 ml wash buffer concentrate with 290 ml ddH_2O). The 1× working solution is stable for at least 1 week from the date of preparation.

Assay Procedure

1. Ensure all reagents and specimen are equilibrated to room temperature (RT, 18-25°C), and swirl all reagents and specimens gently before use. If the entire 96-well plate will not be used, remove surplus strips and wells from the plate frame and place back into the re-sealable foil pouch with desiccant. Seal the pouch

and store at 2-8°C.

2. Number each strip on its end to help identify them should they become detached from the plate frame during the assay. A recommended plate layout is given as follows:



* NC = Negative Control; PC = Positive Control; T# = Sample in duplicate

3. To every well, add 100 µl of control or diluted specimen (diluted serum or neat milk) according to the recommended layout above. It is highly recommended to test specimens in duplicate.
4. Cover the strips with the microplate sealer. Incubate the strips for 30 minutes with shaking at RT.
5. After incubation, remove the plate sealer, discard the contents and wash the strips 5 times with diluted wash buffer, ensuring every well is filled. When washing is completed, tap the strips firmly on absorbent tissue to remove residual wash buffer.
6. Pipette 100 µl of the HRP conjugate solution into each well.
7. Cover the strips with the microplate sealer. Incubate the strips for 15 minutes with shaking at RT.
8. After incubation, wash the strips again as per Step 5.
9. Add 100 µl of TMB solution to each well. Briefly shake to mix, then incubate at RT for 10 minutes in the dark. **Do not cover with the microplate sealer.**
10. Stop the reaction by adding 100 µl of Stop solution to each well in the same order as the TMB solution was added.
11. Measure the optical density (OD) at 450 nm against a reference filter of 620-630 nm within 10 minutes of stopping.

PROCEDURAL NOTES

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in this package insert and with adherence to GLP.
2. It is recommended that all specimens be assayed in duplicate. The average absorbance reading of each duplicate should be used for the calculation of results.

3. Keep all reagents in their original bottles where possible, and avoid unnecessary exposure to light.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Factors that might affect the performance of the assay include proper instrument function/calibration, cleanliness of glassware, quality of distilled or deionised water, accuracy of reagent or sample pipetting, washing technique, incubation time and temperature.
6. Using incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microwells as this could result in lower binding efficiency and higher variance between readings.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming of the reagents.

Quality Control

To assure the validity of the results, each assay must be run with four negative control wells and two positive control wells.

First, the OD values of the Negative controls must be checked. The four Negative OD values should be listed in order of magnitude. If any values are greater than **twice** the value of the **second lowest** Negative OD value, they are considered aberrant and should be discarded. If one value is discarded in this way, use the remaining three to calculate the mean. If two values are discarded, the test is considered invalid and must be re-run.

The following Quality Control criteria must also be met:

The mean OD value of the Positive controls is ≥ 0.500;

The OD value of one Positive control is not greater than double the OD value of the other Positive control.

Interpretation Of Results

Calculate the mean OD value of the negative controls: **NC**

Calculate the mean OD value of each specimen: **OD**

FOR INDIVIDUAL SERUM/MILK SAMPLES:

If $OD \leq 2 \times NC$, result is Negative

If $2 \times NC < OD \leq 2.5 \times NC$, result is Inconclusive

If $OD > 2.5 \times NC$, result is Positive

Negative: Non-infected or unvaccinated.

Inconclusive:

• Repeat the test on the specimen.

• A second sample to be collected and tested in 1-2 weeks.

There may be the following possibilities:

If the re-test is still inconclusive the animal can be considered hardjo negative. This may be caused by cross

reaction by other organisms, e.g. L. saxkoebing.

Positive: Infected/vaccinated.

FOR BULK MILK TANK SAMPLES:

If OD ≤ 2 × NC, result is Negative, Naive or unvaccinated herd

If 2 × NC < OD ≤ 2.5 × NC, result is Low positive. Basically naive, but there may be historical infection/vaccination or cross-reaction with another serovar.

If 2.5 × NC < OD ≤ 12 × NC, result is Medium positive. Status less clear.

If OD > 12 × NC, result is High positive. Herd is likely to be heavily infected and/or vaccinated

Precautions

1. Pease read this package insert completely before using this product
2. Wear gloves while performing this assay and handle reagents and specimens as if they were potentially infectious. If exposure should occur, immediately flush with water.
3. Keep stop solution away from skin and eyes.
4. Follow Good Laboratory Practices (GLP).
5. Discard unused reagents and biological specimens in accordance with local regulations.