BOVINE Tuberculosis ELISA

Enzyme Immunoassay based on microtiter plate for the detection and the quantitative determination of antibodies against ***Mycobacterium bovis*** in serum and plasma of cattle and other animal.

FOR PROFESSIONAL USE ONLY! NOT FOR PERSONAL USE! FOR EXPORT ONLY! NOT FOR SALE IN EUROPE!

Cat.-No.: LIO-TUB06

Version: 190827

FEATURES:

Ready-to-use reagents
Microtiter strips
Detection via Peroxidase / TMB
Procedure time 100 minutes

Manufacturer / Hersteller:

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INTRODUCTION/ FIELD OF APPLICATION

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB), and it has the potential to induce disease in humans. An unknown proportion of TB cases are caused by *M. bovis* (1). Zoonotic TB, caused by *M. bovis*, is present in animals in most developing countries where control measurments are not or sporadically applied, and pasteurization is rarely practiced. In countries where bovine TB is uncontrolled, most infections in human result from drinking or handling contaminated milk (4).

In industrialized countries, veterinary TB control and elimination programs, together with milk pasteurization, have drastically reduced the incidence of disease caused by *M. bovis* in both cattle and humans, but the control programs have not completely eliminated infection in cattle because of wild animal reservoirs (4).

LIONEX has developed a number of highly purified, recombinant mycobacterial antigens that are being used to develop tests for sero-diagnosis of TB. Special attention is being given to cost-effective and rapid tests.

The BOVINE Tuberculosis ELISA kits are suitable for rapid and reliable detection and quantitative determination of antibodies against tuberculosis pathogen in serum samples from cattle or other animal (e.g. badgers, sheep).

ADVANTAGES:

- ⇨ **High sensitivity and specifity**
- ⇨ **High reproducibility**
- ⇨ **Minimal training necessary**
- ⇨ **Individual breakable wells**

KIT CONTENTS:

Σ **96 determinations**

PL	**Microtiterplate** coated with *Mycobacterium bovis* antigens (12 x 8 individual breakable wells).
TMB	**TMB Substrate** Solution: 12 ml, ready to use, containing a solution of tetramethylbenzidine (TMB)
STO	**Stop Solution**: 12 ml, ready to use, 0,2 M H_2SO_4
PV	**Sample Diluent**: 100 ml, ready to use, PBS/BSA buffer, addition of 0.05% 5-bromo-5-nitro-1,3-dioxane.
WB	**Wash Buffer, Concentrate** (10 x): 60 ml, containing PBS buffer with Tween 20, addition of 0.05% 5-bromo-5-nitro-1,3-dioxane.
CON	**Conjugate solution:** 12 mL, ready-to-use, contains of 0.05% 5-bromo-5-nitro-1,3-dioxane.
POS	**POSITIVE standard:** 4 mL, ready-to-use, contains of 0.05% 5-bromo-5-nitro-1,3-dioxane.
WPOS	**Weak POSITIVE standard:** 4 mL,, ready-to-use, contains of 0.05% 5-bromo-5-nitro-1,3-dioxane.
NEG	**NEGATIVE standard:** 4 mL, ready-to-use, contains of 0.05% 5-bromo-5-nitro-1,3-dioxane.

1 instruction manual. **READ CAREFULLY BFORE USE!**

Plastic Bag: Resealable, for the dry storage of non-used strips.

STABILITY AND STORAGE CONDITIONS:

Unopened TEST KIT: until expiry date
Opened TEST KIT: ready-to-use components are stable for min. 6 month. 1 x wash solution: stable for 1 week.

2°C – 8°C
Store at 2-8°

PRINCIPLE OF THE TEST

The BOVINE Tuberculosis ELISA kit is based on the principle of the enzyme immunoassay (EIA). Highly purified specific antigens are bound on the surface of the microtiter strips. Diluted serum samples from cattle ore other animals are pipetted into the wells of the microtiter plate. A binding between the antibodies of the serum and the immobilized antigen takes place. After 45 minutes incubation at 37°C, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use peroxidase conjugate is added and incubated for 30 minutes at 37°C. After a further washing step, the substrate (TMB) solution is pipetted and incubated for **20 minutes** at 37°C, inducing the development of a blue dye in the wells. The colour development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured by an ELISA reader at the wavelength of 450 nm. The concentration of the antibodies is directly proportional to the intensity of the colour.

PRECAUTIONS:

1. Only for in-vitro use! Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Don't work without wearing protective clothing like gloves and lab coat!
2. All kit components should be considered as infectious agents. Wipe off serum and reagent spills with a disinfecting solution (e.g. sodium hypochlorite, 5%)! Dispose residues of kit reagents and samples properly, e.g. by autoclaving.
3. Before use bring all reagents to room temperature (20-30 °C)!
4. Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
5. Pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
6. Avoid contamination of the reagents. Close bottles immediately after removing reagents to avoid oxidation.
7. Use separate disposable pipet tips.
8. Do not use reagents from different kit lots and do not mix reagents of different kits or kit lots with one another.
9. Use all reagents within the expiry period (mentioned on the kit label). After opening, the kit components are stable for 6 months.
10. In accordance with Good Laboratory Practice (GLP) or ISO9001, all laboratory devices employed should be regularly checked for the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
11. Avoid the contact of kit reagents with skin, eye or mucosa, above all the stopping solution and the substrate.

MATERIALS NEEDED BUT NOT PROVIDED

10 µl-, 100 µl- and 500 µl-micro- and multichannel pipets.
Microtiter Plate-Reader (450 nm).
Microtiter Plate-Washer (optional).
Reagent tubes for the serum dilution.
De-ionised or distilled water.
Stop watch.

PREPARATION OF REAGENTS

WB **Washing Solution: Dilute 1:10** with de-ionized or distilled water before use (1 + 9 volume). If crystals precipitate during the cold storage, the concentrate should be warmed up at 37 °C for 15 minutes.

Samples and Sample Preparation:
The serum or plasma samples **can be stored refrigerated (2 - 8 °C) for up to 48 hours**, for a longer storage keep samples at -20 °C. Avoid repeated freezing and thawing of samples. Samples appearing turbid must be clarified prior to use in assay. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test, the samples (**not** the standards) have to be diluted **1:200** with ready-to-use sample diluent **PV** (e.g. 5 µl serum + 995 µl sample diluent **PV**).

PROCEDURE:

Test procedure time 100 min

1. Prepare test reagents: dilute concentrated wash buffer 1:10 with distilled water. Dilute serum samples 1:200 with sample diluent. Equilibrate all kit components to room temperature.

2. Pipet 100 µl each of the diluted samples and ready to use standards into the wells. All samples should be measured in duplicate. Leave one well empty for the substrate blank and incubate for 45 minutes at 37°C.

3. Empty the wells of the plate (dump or aspirate) and wash 3 x with diluted wash buffer **WB** (300 µl/well).

4. Pipet 100 µl each of ready-to-use conjugate **CON** into the wells. Leave empty the well for the substrate blank. Incubate plate at 37°C for 30 minutes.

5. Repeat washing procedure (step 3) and pipet rapidly 100 µl of the ready-to-use TMB substrate **TMB** into all wells, including the well for substrate blank. Incubate plate in the dark for 20 min (37°C)!

6. Terminate the substrate reaction: pipet rapidly 100 µl of the ready-to-use stop solution **STO** into each well.

7. After gentle shaking, wipe the bottom of the plate and measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

INCUBATION STEPS:

Prepare reagents and samples: dilute concentrated wash buffer 1:10 with dest. water, dilute samples 1:200 in Sample diluent (5 µl sample + 995 µl sample diluent).

A: Pipetting: 100 µl diluted samples (1:200) or undiluted standards / well.
INCUBATION for 45 min / 37°C.

B: Washing procedure: 3 x with diluted wash buffer (300 µl / well). **Pipetting:** 100 µl of conjugate / well (ready-to-use).
INCUBATION for 30 min / 37°C.

C: Washing procedure: 3 x with diluted wash buffer (300 µl / well). **Pipetting:** 100 µl of TMB-substrate / well (ready-to-use).
INCUBATION for 20 min / 37°C.

D: Pipetting: 100 µl of Stop-solution / well (ready-to-use)

PRINCIPLE: ENZYME IMMUNO ASSAY

96 well microtiter plate with immobilized antigen cocktail, ready-to-use: prepare samples and test reagents.

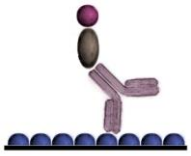
⇓ **STEP A.**

Incubation with samples and standards: TB-specific antibodies from the sample bind to the immobilized antigen.

⇓ **STEP B.**

Washing procedure, pipetting the conjugate

⇓



Incubation with conjugate: Peroxidase-conjugated Protein A/G binds to the antibodies.

⇓ **STEP C.**

Additional washing procedure, pipetting the TMB-substrate: blue colour development
Step D: Addition of stop-solution: colour turns from blue to yellow.

FINALLY: measure OD at 450 nm (optionally 620 nm reference wafe lenght)

LIMITATIONS

The test is evaluated for the detection of antibodies in serum and plasma. For other body fluids the test has not been validated and can yield incorrect results!
POSITIVE test result needs further confirmation! For final diagnosis take all available clinical results of given patient into consideration (X-ray, microscopy, culture results, serology, PCR, clinical signs).
The controls included in the kit are intended to verify if the test was properly carried out. The controls should be run with each batch of samples to check the test performance.

NOTE: NEGATIVE test results do not preclude a possible TB Infection or disease!

INTERPRETATION OF RESULTS

Calculate the mean OD values for the measured absorptions for every sample. Substract the blanc value from every calculated mean absorption. The difference between single values should not exceed 10% for the standard solutions and samples.

BOVINE Tuberculosis ELISA kits can produce NEGATIVE, BORDERLINE or POSITIVE results. **Note that BORDERLINE cases need further confirmations.** Specimens should be rechecked after 2-4 weeks. Diagnosis of TB should not be based only on the results of one assay. Take all clinical data (serological data antibody titres, results of microscopy, culture, skin test e.g.) into consideration to reach the most accurate diagnostic conclusion.

CUT OFF LEVELS FOR BOVINE ELISA KIT

The cut-off level of the BOVINE Tuberculosis ELISA is determined by the evaluation of *Mycobacterium bovis*-negative individuals. But the cut-off level may vary for animals from different regions and countries. **Therefore, the cut-off value should be determined individually by every new user.** We recommend to determine the cut-off by measuring a panel of confirmed negative and positive cases.

We suggest the following cut-off levels for the given species:
CATTLE:
NEGATIVE: OD450 (mean value) < 0,100
BORDERLINE: OD450 = 0,100 – 0,110
POSITIVE: OD450 (mean value) > 0,110
BADGER:
NEGATIVE: OD450 (mean value) < 0,400
BORDERLINE: OD450 = 0,400 – 0,700
POSITIVE: OD450 (mean value) > 0,700

SENSITIVITY AND SPECIFITY:

The specific antigen from *Mycobacterium bovis* is evaluated for cattle. The sensitivity is 75 % for a specifity of 97,8 %.

References

Acha PN, Szyfres B. Zoonotic tuberculosis. In: Zoonoses and communicable diseases common to man and animals. 2nd edition. Washington: Pan American Health Organization/World Health Organization; 1987: Scientific Publication No. 503.

Collins CH, Grange JM. A review. The bovine tubercle bacillus. J Appl Bacteriol 1983;55:13-29.

Cosivi O, Meslin F-X, Daborn CJ, Grange JM. The epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to Africa. Scientific and Technical Review 1995;14:733-46.

Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, Robinson RA, Huchzermeyer HF, de Kantor I, Meslin FX: Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg Infect Dis. 1998 Jan-Mar;4(1):59-70. Review. PMID: 9452399