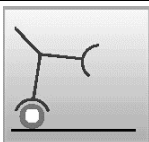
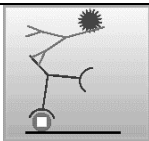


OVERVIEW OF PROCEDURE

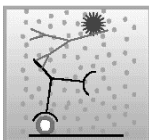
- Dispense 80 µl of specimen diluent and 20 µl both control and investigated sera or milk samples in wells
- Incubate for 20 min at room temperature (18-20°C) (forming complex antigen-antibody)
- Wash 4 times with washing solution



- Dispense 100 µl of conjugate solution in wells
- Incubate for 20 min at room temperature (18-20°C) (forming complex antigen-antibody with conjugate)
- Wash 6 times with washing solution



- Dispense 100 µl TMB substrate in wells
- Incubate for 30 min at room temperature (colouring)
- Stop the reaction by adding 100 µl stop-reagent
- Read the results relative to colours cut off control visually or by measuring the optical density at 450/620 nm



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Diaproph Med
Diagnostics Prophylaxis Medicine

DIA-Brucella ab.combi -V

**Diagnostic immunoenzyme test system for
the detection of antibodies against
Brucella abortus in cattle serum and cow's milk**

192 tests

Product code: T-10206

EXAMPLE FORM

INTENDED USE

Enzyme immunoassay kit is intended for the detection of antibodies to Brucella abortus in bovine serum and milk.

INTRODUCTION

Bovine brucellosis is a zoonotic infectious disease caused by gram-negative bacteria. Despite eradication programs for brucellosis in many parts of the world, infections with B.abortus remains endemic in many bovine populations resulting in serious economic losses.

This disease is accompanied by production of antibodies types IgG1, IgG2 and IgM. IgM type antibodies are detected in the first days of disease, but the antibody quantity is rapidly decreased under infection progressing and antibodies type IgG become prevalent.

Serological diagnostics of brucellosis is used in many countries as a criterion for control and eradication of this disease. Several conventional techniques are used for detection of different antibody isotypes, to determine an animal seropositive to brucellosis it is necessary to use techniques such as Bengal Rose (RBT), agglutination in buffered plate (ABT) or Slow tube agglutination (SAT). The complement fixation test (CFT) is used as confirmatory techniques. These tests suffer some disadvantages: they are time consuming, with low sensitive and difficult to read. The introduction of indirect immunoenzymatic techniques in serological diagnostics of brucellosis allows achieving higher sensitivity and specificity levels in comparison with commonly using conventional techniques. ELISA has already been approved by the Office Internationale des Epizooties (OIE). During the last ten years ELISA becomes the main screening test in the world. The high specificity and sensitivity of the given test, which is rapid and easy at performance, allows carrying out the quality diagnosis under mass investigations of animals.

PRINCIPLE OF PROCEDURE

The main kit components are an immunosorbent and an enzyme-containing conjugate. The immunosorbent is a polystyrene plate whose wells are coated with purified *Brucella abortus* antigens. The conjugate contains horseradish peroxidase (HRP) bound to anti-bovine IgG antibodies.

Investigated serum or milk specimens are put into plate wells. The antibodies against the *Brucella abortus* present in the samples bind to the coated antigens of the solid phase forming antigen-antibody complexes. These complexes are detected using the specific enzyme-containing conjugate. Non-bound components are to be washed out. Following washing procedure, a developer solution containing enzyme substrate and chromogen (tetramethylbenzidine, TMB) are added to wells. The reaction is stopped by a stop-reagent.

The optical density values in wells are determined at 450 nm or visually relative to cut off control colouring and these values being correlated with specific antibody concentration in investigated samples.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit is not subject to freezing. The shelf life of the kit is 12 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

N	Reagents	Presentation
1	Stock washing solution (№1) (concentrate 45X) Phosphate buffer, containing 2.2 % Triton X100.	3 bottles 3 × 25 ml
2	Microelisa strips 6 strips per plate each with 16 wells coated with purified <i>Brucella abortus</i> antigens.	2 plates
3	Solution for sera dilution (№3) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 20 ml
4	Solution for conjugate dilution (№4) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 26 ml
5	Solution for chromogen preparation (№ 5T) Citrate-phosphate buffer, containing 0.016 % hydrogen peroxide.	1 bottle 1 × 14 ml
6	Chromogen (TMB) Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 14 ml

- Cover the plate with adhesive film and incubate at room temperature (18-25°C) for 20 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette 100 µl of the **conjugate solution** into each well.
- Cover the plate with adhesive film and incubate at room temperature (18-25°C) for 20 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 µl of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at 18-25°C for 30 minutes in the dark.
- Add 100 µl of **stop-reagent** into each well to stop colour reaction (maintain the same pipetting sequence and rate used for TMB substrate addition).
- Treatment of results is conducted visually or with reader within 5 min after reaction stopping.

Reading and interpretation results

Visual reading of results

- The microelisa strips in the frame is placed on the white paper in well lighted place and visually compare the colour intensity of contents in wells with investigated specimens with the colour intensity of cut-off controls. If the intensity in one well with cut-off control is distinctly different from others two, this value can be discarded.
- The result is considered **negative** if the colour intensity of investigated specimen is lower than the one in wells with cut-off controls.
- The result is considered **positive** if the colour intensity of investigated specimen is equal or greater than the one in wells with cut-off controls.

Automatic reading of results

- The optical density is defined with the reader at 450 nm (single wavelength) against a blank well; for that purpose include an empty well in the run or at 450/620 nm using a dual wavelength.
- The value of optical density of the negative control is to be not higher than 0.1 optical units (OU), the one of the positive control is to be not lower than 0.6 and the cut-off value in single well is to be not lower than 0.2.
- Calculate the mean value of cut-off. If the optical density in single well with cut-off is greater in twice than the mean cut-off value, this value is discarded.
- The result is considered **negative** if the optical density is lower than the mean cut-off value.
- The result is considered **positive** if the optical density is equal or greater than the mean cut-off value.
- In case of obtaining positive result during investigation of pulls of sera or milk each such sample is to be individually retested (one sample –one well).

- Reagents should not be mixed from different lots during performing test.
- Reagents and samples should be at room temperature (18-25°C) before testing is run. Return the reagents to 2-8°C after use.
- The temperature in room where analysis performing should be in the range 18-25°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.
- TMB substrate (solution for chromogen preparation + chromogen TMB) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.
- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for bringing specimens into wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing is to be strictly performed according to the instructions, as insufficient plate washing leads to incorrect results.

Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.

Follow this procedure in each washing:

- aspirate the wells contents completely into a waste flask;
- then fill the wells completely with washing solution (not less than 350 µl per well) avoiding overflow of buffer from one well to another;
- allow to soak during 30 seconds;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and strip-holder after the last aspiration (e. g. by blotting with absorbent tissue).

* Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the strip-holder with required number of **strips**.
- Pipette 80 µl of the **solution for sera dilution (№3)** into each well.
- Distribute in the wells as follows:
 - wells A1 20 µl of **positive control (C+)**.
 - wells B1 20 µl of **negative control (C-)**.
 - wells C1, D1, E1: 20 µl of **cut off control**.
 - the rest wells : 20 µl of **specimens**.

Carefully repipette mixture in wells.

N	Reagents	Presentation
7	Positive control (C+) Cattle serum containing antibodies to Brucella abortus. Preservatives: 0.1% sodium azide and 0.01% paranitrophenol	1 vial 1 × 0.3 ml
8	Negative control (C-) Cattle negative serum. Preservatives: 0.1% sodium azide and 0.01% paranitrophenol	1 vial 1 × 0.3 ml
9	Cut-off control PBS containing antibodies to Brucella abortus Preservatives: 0.1 % 2-methyl-4-isothiazolin-3-one.	1 vial 1 × 0.9 ml
10	Conjugate (concentrate 50X) Monoclonal antibodies against bovine IgG bound to a horseradish peroxidase (HRP). Preservatives: 0.01% thimerosal.	1 vial 1 × 0.6 ml
11	Stop-reagent 0.5 M sulphuric acid solution.	1 bottle 1 × 25 ml
12	Adhesive film	6 items

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vials for reagent preparation (glass or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- hydrogen peroxide 6% or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 µl) with disposable tips;
- automatic multi-channel pipettes (50-300 µl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with dual wavelength 450/620);
- biohazard waste containers for potentially contaminated materials.

* Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens into wells.
- All reagents included in the kit are intended for "in vitro" diagnostic use.

- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.
- Do not pipette by mouth.
- All liquid biohazardous wastes are to be treated by hydrogen peroxide 6% at room temperature during 3 hours.
- All solid wastes are to be stored in a special container and autoclaved during 1 hour at 120°C.
- Instruments, equipment and also working surfaces are to be treated by with 70° ethanol.

SPECIMEN PREPARATION

Serum specimens are to be stored at 2-8°C for 72 hours. If necessary these specimens may be frozen (more than two freezing-thawing procedures are not allowed) at temperature below -20°C.

Milk samples are to be stored at 2-8°C for 24 hours. Meanwhile the fat will be on the top of the milk if not it is to be centrifuged at 1500 g for 15 minutes. A defatted milk must be used for investigation. Particulates of fat cause false negative results.

All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

It is allowed investigating serum or milk specimens in pull including up to 10 ones.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-25°C) before beginning the assay and can be remained at room temperature during testing. After use return reagents to 2-8°C.

Reagents preparation

Microelisa strips

Open the pack and remove the plate. Return unused strips in the pack. Reseal the pack and return to 2-8°C.

The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **Stock washing solution (№1)** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37 °C.

Dilute the **stock washing solution (№1)** with distilled or deionised water (see chart below) shake intensively.

Washing solution is stable for 10 days at 2-8 °C.

Number of wells	Stock washing solution (№1)	Distilled water
16	6 ml	270 ml
32	8 ml	360 ml
48	12 ml	540 ml
64	16 ml	720 ml
80	20 ml	900 ml
96	25 ml	1125 ml

Conjugate solution

Dilute in the ratio 1:50 the **conjugate** with **solution for conjugate dilution (№4)** (see chart below) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Conjugate solution is stable for 2 weeks at 2-8 °C.

Number of wells	Conjugate (concentrate 50X)	Solution for conjugate dilution (№4)
16	40 µl	2 ml
32	80 µl	4 ml
48	120 µl	6 ml
64	160 µl	8 ml
80	200 µl	10 ml
96	240 µl	12 ml

TMB substrate

To prepare **TMB substrate**, combine the required amount of **chromogen TMB** in a clean vial in equal parts with **solution for chromogen preparation (№5T)** according to the number of wells being run (see chart below). Mix well. TMB substrate is to be colourless before use.

The TMB substrate is to be kept away from light and no solutions contact with metals or metal ions is allowed.

The substrate solution is to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-25 °C) if kept in the dark.

Number of wells	Chromogen TMB	Solution for chromogen preparation (№5T)
16	1 ml	1 ml
32	2 ml	2 ml
48	3 ml	3 ml
64	4 ml	4 ml
80	5 ml	5 ml
96	6 ml	6 ml

Procedural notes

Authenticity of results depends on correct execution following instructions:

- Reagents should not be used beyond the expiry date shown on the package label.