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User's Manual

Brucella Ab ELISA

Enzyme Immunoassay for the detection of antibodies against polysaccharide epitopes of Brucella abortus in serum and milk samples

RUO

REF	DE2497	DE5487
Σ	96	5 x 96

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Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1 INTRODUCTION

Despite eradication programmes for brucellosis in many parts of the world, infection with Brucella abortus remains endemic in many cattle populations resulting in serious economic losses.
Serological identification of Brucella infected cattle is routinely performed by screening serum samples for antibodies against bacterial agglutinating antigens. These tests suffer some disadvantages: they are time consuming, insensitive and difficult to read. To detect antibodies in milk samples more sensitive test systems are required.
This monoclonal antibody based ELISA test system is intended to use as a rapid screening test for the detection of Brucella antibodies in serum and milk samples of infected cattle.

2 INTENDED USE OF THE TEST KIT

This diagnostic test is intended to identify antibodies against sugar antigens of Brucella abortus, in serum and milk samples.
In contrast to test systems which make use of agglutinating bacterial antigen, this partial monoclonal based ELISA has a very high sensitivity and specificity (according to SAT, EC and Weybridge standards).

3 PRINCIPLE OF THE TEST KIT

Diluted milk or serum samples are added to the pre-coated wells. After incubation and appropriate washing a monoclonal anti-bovine IgG antibody conjugate is added and the plates are again incubated. After appropriate washing, substrate is added. After several minutes the colour reaction is stopped and the plates are immediately read at 450 nm.

4 CONTENTS

	DE2497 (96 wells)	**DE5487 (5x96 wells)**
Microtiter plates coated with Brucella polysaccharide antigen	1 x 96 well	5 x 96 well
Conjugate Buffer	1 x 15 mL	1 x 60 mL
Concentrated HRPO conjugate Dilute 1:100 in conjugate buffer	1 x 0.3 mL	1 x 1.5 mL
Inactivated **positive control** standardized to 200 EIU/mL (freeze-dried)	1 x	1 x
Inactivated **negative control** (freeze-dried)	1 x	1 x
Wash solution 200 x concentrated Dilute in deionized water before use!	1 x 20 mL	1 x 50 mL
ELISA buffer	1 x 22 mL	2 x 40 mL
Substrate buffer A	1 x 7 mL	1 x 40 mL
Substrate buffer B	1 x 7 mL	1 x 40 mL
Stop solution	1 x 8 mL	1 x 50 mL

5 HANDLING AND STORAGE OF KIT AND SPECIMENS

The kit should be stored at 4 °C.
An open packet should be used within 10 days.
Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date.

Samples may be used fresh or may be kept frozen below -20 °C before use.
Fresh samples can be used without restriction. Addition of 0.1% sodium azide to the samples has no influence on the results.
Avoid repeated freezing and thawing as this increases non-specific reactivity

Milk samples- undiluted; For optimal **sensitivity** pooled milk samples can be tested undiluted. To avoid false positive reactions defatted samples must be used Centrifuge the milk samples for 15 minutes at 2500 g and take a sample from below the fat layer.

Milk samples- diluted: For optimal **specificity i**ndividual milk defatted samples should be diluted 1:4 in ELISA buffer. Pooled milk samples, collected up to 25 individual animals, can also be tested in a 1:2 dilution. The use of diluted milk samples guarantees minimum false positive reactions

Serum samples: Individual serum samples should be diluted 1:100 in ELISA buffer.

6 WASH PROTOCOL

In ELISAs, uncomplexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below.

Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing

1. Empty each well by turning the microtitre plate upside down, followed by a firm vertical movement.
2. Fill all the wells with 250 µL washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times.
4. Turn the plate upside down and empty the wells by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

When using automatic plate washing equipment, check that all wells can be aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL

1. Reconstitute the positive and negative controls in 0.5 mL aqua bidest. (not provided).
   Make a 2-fold dilution series of the Brucella ELISA standard provided (1:30, 1:60, 1:120, 1:240, 1:480, 1:960) in the first six wells of the first row (A, B, C, D, E, F) of a round bottomed microtiter plate.
   Buffer is only added to well G1.
   Make in H1 a 1:100 dilution of the negative control.
2. Diluted serum samples (1:100) are added to the other wells of the round bottom plate.
   For milk samples, add 100 µL of milk, preferably undiluted or 1:4 diluted to the wells of the round bottomed plate.
3. Transfer 100 µL of controls and samples to the coated microtiter plate.
4. Seal the microtiter plate and incubate for 60 minutes at 37 °C.
5. Wash as described in chapter 6.
   (Dilute the washing fluid 1:200 in aqua bidest. before use)
6. Dilute the concentrated HRPO conjugate 1:100 in conjugate buffer.
7. Immediately dispense 100 µL antibody conjugate to all wells.
8. Seal and incubate 1 hour at 37 °C.
9. Wash as described in chapter 6.
10. Mix equal parts of buffer A and buffer B together with gentle shaking. Prepare immediately before use!
    Dispense 100 µL substrate solution to each well.
    Incubate 10-12 minutes at room temperature (21 °C).
11. Add 50 µL stop solution to each well (mix well).
12. Read the absorbance values immediately (within 10 min!) at 450 nm. Use 620 nm as a reference.

8 PRECAUTIONS

- Handle all biological materials as though capable of transmitting Brucella (humane pathogene).
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or if swallowed. Observe care when handling the substrate.
- Do not use components past their expiry date and do not inter-mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtitre plate and prevent it from damage and dirt.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the mean OD of the negative control should be lower than 0.150 (450 nm).
The 1:30 dilution of the Brucella ELISA standard provided should give an OD (450 nm) of ≥ 1.000.

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

a. **Qualitatively:** positive or negative
b. **Quantitatively:** ELISA units which can be transformed into agglutination units.

a. **qualitatively:**

A sample is scored negative if the OD is lower than 2 x OD of the negative control.
A sample between 2 x and 3 x the OD of the negative control is considered weak positive.
A sample above 3 x the negative OD is considered to be positive.

b. **quantitatively:**

The value in E.C.-units of the samples can be calculated by comparison to their OD-values with a curve which is constructed from the OD-values of the dilutions of the standard (Y-axis) and their corresponding values in units (200; 100; 50; 25, etc, X-axis) on log/log paper.
With this graphic presentation it is possible to determine the value in units of the samples.
According to EC standards values below 12.5 IU are considered negative.
Values above 32 IU are considered positive.
Values between 12.5 and 32 IU are considered doubtful.

The entire risk as to the performance of these products is assumed by the purchaser. DEMEDITEC shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungs-zwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità