DRG® Brucella ELISA (bovine) (EIA-2497)

Revised 28 July 2010 rm (Vers. 6.0) **For Veterinary Use Only**

Please use only the valid version of the package insert provided with the kit.

For Veterinary use only!

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 preformed 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.

INTENDED USE OF THE BRUCELLA 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).

STANDARDIZATION

To standardize the brucella ELISA, positive and negative standards are tested which resulted in a new brucella ELISA standard.
The correlation with international used agglutination standards is approximately
1 ELISA-IU corresponds with 1 agglutination IU.

PRINCIPLE OF THE BRUCELLA-TEST

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.

CONTENTS OF THE BRUCELLA TEST KIT

- 1 x 96 well **microtiter plates coated** with brucella antigen
- 1 x 15 ml **conjugate buffer**
- 1 x 0,3 ml **concentrated HRPO conjugate**, dilute 1:100 in conjugate buffer
- 1 x inactivated **positive control** standardized to 200 EIU/ml (freeze-dried).
- 1 x inactivated **negative control** (freeze-dried)
- 1 x 20 ml **wash solution 200 x concentrated** (Dilute in deionized water before use!)

- 1 x 22 ml **ELISA buffer**.
- 1 x 7 ml **substrate buffer A**
- 1 x 7 ml **substrate buffer B**
- 1 x 8 ml **stop solution**.

STORAGE OF KITS

The kit should be stored at 4-8ºC. An unopened package can be used until the expiry date. An opened package can be used, if the requirements, mentioned in the validation (See 11) are fulfilled. If not fulfilled the test can no longer be used. Avoid repeated freezing and thawing as this increases non-specific reactivity. Positive and negative controls may be stored after reconstitution in aliquots at –20°C and used until the expiry date.

HANDLING AND STORAGE OF SPECIMENS

Fresh samples can be used without restriction. Addition of 0.1% sodium azide to the samples has no influence on the results.
For prolonged storage, samples should be frozen as soon as possible and stored at -25ºC until use.
Avoid repeated freezing and thawing as this increases non-specific reactivity and decreases the end point titer.

I. 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.

II. Milk samples - diluted

For optimal specificity individual milk samples should be tested at a 1:4 dilution.
Pooled milk samples, collected from up to 25 individual animals, can also be tested in at a 1:2 dilution.
The use of diluted milk samples guarantees minimum false positivity.

III. Serum samples

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

WASH PROTOCOL

In ELISA's, between each immunological incubation step un-complexed components have to be removed efficiently. 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 advised to carefully follow the washing procedures outlined below.
Both manual washing and washing with automatic equipment can be performed. (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.

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 min. at 37ºC.
5. Wash as 1. (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 ºC.
9. Wash as 1.
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 min. at room temperature (21ºC).
11. Add 50 µl stop solution to each well (mix well).
12. Read the absorbance values immediately (within 10 min.!) at 450 nm. Use 620nm as a reference.

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 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 intermix 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.

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.

INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

qualitatively: positive or negative
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.

quantitatively: ELISA units which can be transformed into agglutination units.
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. DRG shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

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Symbols used with DRG 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
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	For veterinary use only				
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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