Instructions for Use

Brucella Ab (Bovine) ELISA

VET

REF	EIA-2497	EIA-5487
Σ	96	480

DRG Instruments GmbH, Germany
Frauenbergstraße. 18, D-35039 Marburg
Phone: +49 (0)6421-1700 0, Fax: +49 (0)6421-1700 50
Website: www.drg-diagnostics.de
E-mail: drg@drg-diagnostics.de

Distributed by:

DRG International, Inc., USA
841 Mountain Ave., Springfield, NJ 07081
Phone: (973) 564-7555, Fax: (973) 564-7556
Website: www.drg-international.com
E-mail: corp@drg-international.com

(March 2018)

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Table of Contents

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 bovis, 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, E.C. and Weybridge standards).

3 PRINCIPLE OF THE TEST KIT

Diluted milk or serum samples are added to the pre-coated wells.

- **Qualitative**

The serum sample is added (diluted 1:150) to the wells of the coated plate.

The milk sample is added (1:2) to the wells of the coated plate.

- **Quantitative**

The serum sample also can be titrated using a 3-step dilution, starting with a dilution 1:30 (→ 1:90 → 1:270 → 1:810).

The milk sample also can be titrated using a 3-step dilution, starting with a dilution 1:2 (→ 1:6 → 1:18 → 1:54)

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 color reaction is stopped and the plates are immediately read at 450 nm.

4 CONTENTS

EIA-2497	EIA-5487	
12 x 8	5 x 12 x 8	Microtiter strips coated with Brucella polysaccharide antigen
1 x	5 x	Strip holder
1 x 18 mL	2 x 40 mL	ELISA buffer (green cap)
1 x 12 mL	1 x 60 mL	HRPO conjugated anti-species antibodies (red cap)
1 x 0,5 mL	1 x 0,5 mL	Inactivated positive control (freeze-dried) (purple cap)
1 x 1,0 mL	1 x 1,0 mL	Negative control (freeze dried) (silver cap)
1 x 20 mL	1 x 60 mL	Wash-solution (200x concentrated) (black cap), **diluted in de-ionized water before use!**
1 x 8 mL	1 x 40 mL	Substrate A (white cap)
1 x 8 mL	1 x 40 mL	Substrate B (blue cap)
1 x 8 mL	1 x 60 mL	Stop-solution (yellow cap)
1 x	5 x	Plastic cover seal
1 x	1 x	Instructions for Use

4.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Precision pipette 0.1 - 5 µL
- Precision pipette 10 - 200 µL
- Precision pipette 200 - 1000 µL
- Pipette tips and clean containers/tubes
- ELISA plate reader

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 4 °C.

An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20 °C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity.

6 WASH PROTOCOL

In ELISA's, un-complexed 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 result.

Manual washing

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

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are 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 5 washing cycles.

7 PREPARATIONS

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 minutes at room temperature (± 21 °C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4 °C - 8 °C immediately after use.

8 TEST PROTOCOL QUALITATIVE - SERUM

Before starting this test read "Preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest. (5 MΩ water) !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **positive control** (purple cap) in 0.5 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.

3. Reconstitute directly before use the **negative control** (silver cap) in 1.0 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.

4. Dilute the **positive control 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL buffer to well **1A**, add 10 µL of the positive control to the well **1A** and mix well.
   - Add 140 µL buffer to well **1B**, add 10 µL of pre-dilution well **1A** in the well **1B** and mix well
   (only transfer this dilution to the coated plate at step 8).

5. Dilute the **negative control 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL buffer to well **1C**, add 10 µL of the negative control to the well **1C** and mix well.
   - Add 140 µL buffer to well **1D**, add 10 µL of pre-dilution well **1C** in the well **1D** and mix well
   (only transfer this dilution to the coated plate at step 8).

6. Dilute **each sample 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL buffer to well **1E**, add 10 µL of the sample to the well **1E** and mix well.
   - Add 140 µL buffer to well **1F**, add 10 µL of pre-dilution well **1E** in the well **1F** and mix well
   (only transfer this dilution to the coated plate at step 8).

7. Take 2 wells as substrate controls add only 140 µL ELISA buffer (green cap) to these well.

---

8. Transfer 100 µL of all dilutions to the Brucella coated microtiter strips.
9. Seal and incubate for 60 minutes at 37 °C.
10. Wash the plate 5x according to the wash protocol see chapter 6.
11. Dispense 100 µL of the diluted HRPO conjugated anti-species antibody to all wells.
12. Seal and incubate for 60 minutes at 37°C see chapter 6.
13. Wash the plate according to the wash protocol.
14. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking. **Prepare immediately before use! Prepare only the amount needed. Substrate can only be used for 1 hour after being mixed**
15. Dispense 100 µL substrate solution to each well.
16. Incubate 10 - 13 minutes in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C). Make sure the negative control does not become too dark.
17. Add 50 µL stop solution to each well; mix well.
18. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round bottom microtiter plate second step can be done directly in the coated ELISA plate.

9 TEST PROTOCOL QUANTITATIVE - SERUM

Before starting this test read “Preparations”

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 MΩ) water !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Reconstitute directly before use the **positive control** (purple cap) in 0.5 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.
3. Reconstitute directly before use the **negative control** (silver cap) in 1.0 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.
4. Make a **pre-dilution of the positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to well **1A** and add 20 µL of the positive control to the well **1A**.
5. Make a **pre-dilution of the negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to well **1B** and add 20 µL of the negative control to the well **1B**.
6. Make a **pre-dilution of each sample** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to well **1C** and add 20 µL of the sample to the well **1C**.
7. Take 2 wells as substrate controls add only 140 µL ELISA buffer (green cap) to these well.

---

8. Add for dilution of the **positive control** 125 µL buffer to well **1A**.
   And 100 µL to **1B, 1C, 1D** of the coated microtiter strip.
9. Add for dilution of the **negative control** 125 µL buffer to well **1E**.
   And 100 µL to **1F, 1G, 1H** of the coated microtiter strip.
10. Add for dilution of the samples 125 µL buffer to the other well **2A** and **2E**.
    And 100 µL to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.
11. Make a 3-step dilution of the **positive control** in the coated microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL positive control from step 4 to the well **1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1B**
    - Mix well and transfer 50 µL to the next well **1C**
    - Mix well and transfer 50 µL to the next well **1D**
    - Mix well and discard 50 µL.
12. Make a 3-step dilution of the **negative control** in the coated microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL negative control from step 5 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well and transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the next well **1H**
    - Mix well and discard 50 µL.
13. Make 3-step dilution of **each sample** in the coated microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL of each sample from step 6 to the well **2A and/or 2E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **2B and/or 2F**
    - Mix well and transfer 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.
14. Dispense 100 µL of the substrate control of step 7 to the last 2 wells of the microtiter strip.
15. Seal and incubate for 60 minutes at 37 °C.
16. Was the plate 5x according to the wash protocol see chapter 6.

17. Dispense 100 µL of the diluted HRPO conjugated anti-species antibody to all wells.
18. Seal and incubate for 60 minutes at 37 °C.
19. Wash the plate according to the wash protocol see chapter 6.
20. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1 hour after being mixed**
21. Dispense 100 µL substrate solution to each well.
22. Incubate 10 - 13 minutes in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C). Make sure the negative does not become too dark.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

10 TEST PROTOCOL QUALITATIVE - MILK

Before starting this test read Preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ) water !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **positive control** (purple cap) in 0.5 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.

3. Reconstitute directly before use the **negative control** (silver cap) in 1.0 mL aqua bidest. (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.

4. To avoid false positive reactions **defatted** samples must be used. Centrifuge the milk samples for 15 minutes at 2500g and take a sample from below the fat layer.

5. Dilute the **positive control 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example*:* - A pre-dilution is needed:
   - Add 90 µL buffer to well **1A**, add 10 µL of the sample to the well **1A** and mix well.
   - Add 140 µL buffer to well **1B**, add 10 µL of pre-dilution well **1A** in the well **1B** and mix well
   (only transfer this dilution to the coated plate at step 9).

6. Dilute the **negative control 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example*:* - A pre-dilution is needed:
   - Add 90 µL buffer to well **1C**, add 10 µL of the sample to the well **1C** and mix well.
   - Add 140 µL buffer to well **1D**, add 10 µL of pre-dilution well **1C** in the well **1D** and mix well
   (only transfer this dilution to the coated plate at step 9).

7. Dilute **each sample 1:2** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: Add 70 µL buffer to well 1C, add 70 µL sample to well 1C and mix well.

   Pooled samples should be tested undiluted.

8. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these well.

---

9. Transfer 100 µL of all dilutions to the Brucella coated microtiter strips.
10. Seal and incubate for 60 minutes at 37 °C.
11. Wash the plate 5x according to the wash protocol see chapter 6.
12. Dispense 100 µL of the diluted HRPO conjugated anti-species antibody to all wells.
13. Seal and incubate for 60 minutes at 37 °C.
14. Wash the plate according to the wash protocol see chapter 6.
15. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1 hour after being mixed**
16. Dispense 100 µL substrate solution to each well.
17. Incubate 10-13 minutes in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C). Make sure the negative control does not become too dark.
18. Add 50 µL stop solution to each well; mix well.
19. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the pre-dilution step is done in round bottom plate, second step can be done directly in the coated ELISA plate all other steps are done before pipetting directly into the ELISA plate.

11 TEST PROTOCOL QUANTITATIVE - MILK

Before starting this test read “Preparations”

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 MΩ) water !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Reconstitute directly before use the **positive control** (purple cap) in 0.5 mL aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.
3. Reconstitute directly before use the **negative control** (silver cap) in 1.0 mL aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use. Avoid freeze and thaw cycles.
4. To avoid false positive reactions **defatted** samples must be used.
   Centrifuge the milk samples for 15 minutes at 2500g and take a sample from below the fat layer.
5. Make a pre-dilution of the **positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to **row 1A** and add 20 µL of the positive control to the well **1A.**
6. Make a pre-dilution of the **negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to **row 1B** and add 20 µL of the negative control to the well **1B**.
7. Take 2 wells as substrate controls add only 140 µL ELISA buffer (green cap) to these well.

---

8. Add for dilution of the **positive control** 125 µL buffer to **row 1A** and
   100 µL to **1B, 1C, 1D** of the coated microtiter strip.
9. Add for dilution of the **negative control** 125 µL buffer to **row 1E a**nd
   100 µL to **1F, 1G, 1H** of the coated microtiter strip.
10. Add for dilution of the **samples** 75 µL buffer to the other **row 2A and 2E.**
    And 100 µL to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.
11. Make a 3-step dilution of the **positive control** in the coated microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL positive control from step 2 to the well **1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.
12. Make a 3-step dilution of the **negative control** in the coated microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL negative control from step 3 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well and transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the next well **1H**
    - Mix well and discard 50 µL.
13. Make 3-step dilution of **each defatted milk sample** in the coated microtiter strip, starting 1:2 → 1:6 → 1:18 → 1:54.
    Example: - Dispense 75µL of each sample to the well **2A and/or 2E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **2B and/or 2F**
    - Mix well and transfer 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.

    Pooled samples should be diluted; undiluted → 1:3 → 1:9 → 27.
14. Dispense 100 µL of the substrate control of step 7 to the last 2 wells of the microtiter strip
15. Seal and incubate for 60 minutes at 37 °C.

16. Was the plate 5x according to the wash protocol see chapter 6.
17. Dispense 100 µL of the diluted HRPO conjugated anti-species antibody to all wells.
18. Seal and incubate for 60 minutes at 37 °C.
19. Was the plate according to the wash protocol see chapter 6.
20. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1 hour after being mixed**
21. Dispense 100 µL substrate solution to each well.
22. Incubate 10 - 13 minutes in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C). Make sure the negative does not become too dark.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

12 PRECAUTIONS

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not 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 microtiter plate and protect it from damage and dirt.

13 VALIDATION OF THE TEST

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 1.000
  - The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.150

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 1.000 OD units (450 nm) and give an endpoint titer of ≥ 90.

The negative control should be lower than 0.400 OD units (450 nm) and give an endpoint titer of ≤ 30.

14 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio < 0.4 are negative
  - Specific antibodies to Brucella could not be detected.
- A sample with the S/P ratio ≥0 .4 are positive
  - Specific antibodies to Brucella were detected.

Quantitative: End point titre

The ELISA titre can be calculated by constructing a curve, using cut-off line
(as example milk dilution 1:2 – 6 – 18 – 54 – 162 – 486 etc. total 8 dilutions of 3 steps
for serum 1:30 – 90 – 270 etc.)

OD on Y-axis and Titre on X-axis.

ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:30.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
VET	For research use only				
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité