

Manufacturer:  
SYNBIOTICS EUROPE SAS  
2, rue Alexander Fleming  
F- 69367 Lyon, Cedex 07  
France

## SERELISA<sup>®</sup> BTV Ab Mono Blocking

**KIT FOR THE DETECTION OF BLUE TONGUE VIRUS  
ANTIBODIES IN RUMINANTS  
IN SERUM AND PLASMA SAMPLES  
(INDIVIDUAL)**

### BLOCKING IMMUNOENZYMATIC TECHNIQUE

192 single well reactions

#### I. PRINCIPLE OF THE TEST

The SERELISA<sup>®</sup> BTV Ab Mono Blocking is a sensitive test for the detection of antibodies to the major group antigen of bluetongue (VP7). This assay is usually used to test serum or plasma samples from ruminants (especially cattle, sheep and goats) for certification for freedom from infection for regulatory and commercial purposes, especially for export of live animals. This test can also monitor the vaccination in process of animals.

A positive result in this test indicates that infection has occurred with one or more of the 25 serotypes of BTV, but it does not indicate which of the serotypes has been responsible for the infection. The test is more sensitive than the agar gel immunodiffusion (AGID) test, and is also more specific than that test, in that it does not detect antibodies elicited by the other orbivirus serogroups such as the EHD and Palyam groups. The test has the added advantage that it is not species dependent and sera from sheep, cattle or other animals likely to be infected with Bluetongue virus can be tested concurrently.

There are three steps:

1. Each serum or plasma sample is placed in a well sensitised with the BTV Antigen (Ag). Antibodies present in the sample bind specifically onto the viral antigen coated to the well.

2. An anti-VP7 monoclonal antibody (MAb) conjugate peroxidase is added. This conjugate binds to a BTV specific epitope, fixes onto the free antigenic sites forming a complex:  
(Ag) - (MAb anti-VP7 - peroxidase).

3. Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by the addition of a substrate which is transformed into a coloured product. The corresponding optical densities are read and interpreted as follow:

- In the absence of antibodies in the sample, an intense coloured reaction is observed due to the reaction of the enzyme conjugate, which is bound to the free antigen fixed to the solid support.

- In the presence of anti-BTV antibodies in the sample, less enzyme conjugate is bound to the antigenic sites on the solid support and thus the coloured reaction is diminished.

#### II. KIT COMPOSITION AND CONSERVATION

| REAGENT NATURE   | RECONSTITUTION AND CONSERVATION  |
|--|--|
| 2 microplates containing 6 strips of 16 wells sensitised with BTV Ag     | Use within 4 weeks after opening of the sachet which must be closed after use.         |
| Conjugate:<br>BTV specific MAb - peroxidase (CJ)<br>(100 X concentrated) | To be diluted 100 times in the CD. <b>To use the diluted solution within 2 hours.</b>  |
| Buffered peroxidase substrate (PS)                                       | Ready-to-use.  |
| Negative control (NC)  | Ready-to-use.  |
| Positive control (PC)  | Ready-to-use.  |
| Sample diluent pink color (SD)   | Ready-to-use.  |
| Wash solution (W)<br>(10X concentrated)                                  | Dilute 10 times in distilled or demineralised water. Use within 5 days after dilution. |
| Conjugate diluent blue color (CD)  | Ready-to-use.  |
| Stop solution (S)<br><b>Attention caustic!</b>                           | Ready-to-use.  |
| Adhesive films   | 6  |

**Note :** Store diluted reagents at +5 ± 3°C and use as mentioned above.

#### III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 10 to 1000 µl.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.

#### IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

1. Do not mix or associate reagents from kits with different batch numbers
2. Do not use reagents after the expiry date.
3. Place all reagents at laboratory temperature for at least 1 hour prior to use. **Caution:** only the reagents to be used in the following step are concerned.
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
9. It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk and safety phrases:

- S28: After contact with skin, wash immediately with plenty of *water*  
 S36: Wear suitable protective clothing  
 R35: Causes severe burns.  
 S 7: Keep container tightly closed.  
 S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.  
 S30: Never add water to the product.  
 S45: In case of accident or if you feel unwell, seek medical advice immediately.  
 S46: If swallowed, seek medical advice immediately and show this container or label

## V. SAMPLES

The reaction is performed on individual sera and plasma diluted 1:5. Samples should be stored as follows:

| Samples                     | Cold (+5°C) | Freeze (-20°C) | Lab Temperature (+20°C) |
|-----------------------------|-------------|----------------|-------------------------|
| Individual serum and plasma | max. 7 days | Yes            | No                      |

## VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each plate or partially used plate.

### A. PRELIMINARY STEPS

1. Carefully set up the distribution and identification of controls and samples.

2. Prepare the samples to be tested. 1:5 dilutions in sample diluent (SD) should be performed either before in hemolysis tubes, in a blank microplate or directly in the test wells.

### B. TEST PROCEDURE

#### I - CONTROL AND SAMPLE DISTRIBUTION

##### 1. Control distribution:

After shaking the vials, dispense 100 µl of ready-to-use negative control (NC) to wells A1 and A2, and ready-to-use positive control (PC) to wells B1 and B2.

##### 2. Sample distribution:

- Place 100 µl of the 1:5 diluted samples per well.
- For diluting directly in the wells, place 80 µl of sample diluent (SD) plus 20 µl of sample in the well.
- Samples may be tested individually or in duplicate.
- Strips should always be placed on the frame so that both washer and reader can be used.
- Cover the wells with adhesive film, cut to the necessary length by the number of strips used.
- For automate use, the adhesive film is not necessary.
- Mix by gentle shaking the plate manually or by using a plate agitator.

##### 3. Sample incubation:

Incubate for 45 ± 5 min at room temperature (+23 ± 5°C).

**!!! DO NOT WASH AFTER INCUBATION  
DO NOT REMOVE LIQUID**

#### II – ADDITION OF CONJUGATE

##### 1. Preparation of conjugate:

Prepare the conjugate solution by diluting the concentrate (CJ) 1:100 in the conjugate diluent (CD); 2 ml are needed for one strip, meaning 20 µl of CJ in 1.980 ml of CD.

**Caution:** use the diluted solution within 2 hours.

##### 2. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

For automate use, the adhesive film is not necessary.

Mix by gentle shaking the plate manually or by using a plate agitator.

##### 3. Incubation of conjugate:

Incubate for 30 ± 5 min at room temperature (+23 ± 5°C).

### WASHING:

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralised water.

Carefully remove the adhesive film, empty the wells and wash 4 times.

**Sufficient and homogenous washing of the plate is absolute crucial to afford valid results. The use of an automated plate washer or a squirt bottle with spout tube is highly recommended.**

#### III – REVELATION

##### 1. Addition of the substrate:

Add 100 µl of peroxidase buffered substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or use a plate agitator to ensure correct mixing.

##### 2. Incubation of substrate:

15 ± 2 min at room temperature (+23 ± 5°C) shielded from light.

##### 3. Addition of Stop Solution:

Add 50 µl of stop solution (S) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubble occurs in the wells.

##### 4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

## VII. TEST VALIDATION

Valid SERELISA® BTV Ab Mono Blocking result is obtained when:

- the average optical density (OD) value of the negative controls (A1, A2) is > 0.6 and
- the P/N ratio is < 0.3 and it is calculated as follow:

$$P / N = \frac{\overline{DO(PC)}}{\overline{DO(NC)}}$$

## VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

- Calculate for each sample the S/N ratio
- Calculate the average of the OD if samples are tested several times

This S/N ratio is calculated as follows:

$$S / N = \frac{DO(S)}{DO(NC)}$$

### RESULTS

|                 | S/N 0.35 | S/N 0.45 |
|-----------------|----------|----------|
| Serum or plasma | +        | + / -    |

- Any **serum** presenting a S/N ratio ≤ 0.35 is considered as **positive**.
- Any **serum** presenting a S/N ratio > 0.45 is considered as **negative**.

### Doubtful Zone:

Any individual serum, plasma which presents a S/N ratio situated in a zone from 0.35 to 0.45 is considered as **doubtful** and should be re-tested. If the doubtful result is confirmed, a second test on a different sample from the same animal is recommended.

### REMARKS:

For a non-vaccinated animal, a positive result by the test indicates that infection has occurred with one or more of the 25 serotypes of BTV, but it does not indicate which of the serotypes has been responsible for the infection.

Should you have any question, please contact us:  
SYNBIOTICS EUROPE - 2 rue Alexander Fleming  
69367 LYON Cedex 07 – France  
Tel : +33 4.72.76.11.11 - Fax : +33 4.72.76.11.10  
www.synbiotics.com techsupport@synbiotics.fr

FOR VETERINARY USE ONLY /  
FOR IN VITRO USE ONLY

Classified facilities for the protection of the environment