Infectious Bovine Rhinotracheitis (IBR-Ab)  
SVANOVIR™  
ELISA test for the detection of IBR antibodies in serum and milk

**General information**

Infectious Bovine Rhinotracheitis (IBR) is a severe respiratory herpesvirus infection in cattle characterized by tracheitis, rhinitis and fever. The virus may also cause conjunctivitis, infectious pustular vulvovaginitis (IPV), balanoposthitis, abortions, and in rare cases encephalitis. IBR is transmitted horizontally by contact with respiratory, ocular, and reproductive secretions. IBR also acts as an immunosuppressive, predisposing individuals to secondary bacterial infections. The clinical manifestations of the disease affect feed efficiency, milk yield, and reproduction. Economic losses can be high due to these factors and therefore herds should be monitored for the presence of infection. In Switzerland, an indirect Enzyme Linked Immunosorbent Assay (ELISA) of serum and milk samples has been introduced very early, resulting in a successful country-wide control by culling the infected cattle.

**Interpretation**

It is recommended to retest all samples with a positive reaction in an ELISA with control antigen, to eliminate any doubtful reacting samples.

**Contents**

- IBR antigen coated microtitre plates, (sealed and stored dry)
- Lyophilized HRP Conjugate (horseradish peroxidase conjugated antibovine IgG monoclonal antibodies)
- PBS-Tween Solution 20 x concentrate
- Substrate Solution – (tetramethylbenzidine in substrate buffer containing H₂O₂) – STORE IN THE DARK!
- Stop Solution – Contains sulphuric acid – CORROSIVE!

A. Positive Control Serum – 0.05% merthiolate

**References**


**Manufacter**

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This manual covers the following IBR-Ab ELISA kit: Article number 10-2100-50
**Materials needed but not provided**
1. Precision pipets (range from 4 to 200 µl)
2. Disposable pipet tips
3. Distilled water
4. Wash bottle
5. 1 container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter

**Specimen information**
- **Serum:** 4 µl of blood serum or plasma is needed for each sample well. Fresh, refrigerated, or previously frozen serum or plasma may be tested.
- **Milk:** 100 µl of skim milk is required for each sample well. Milk samples must be centrifuged for 15 minutes at 2000 x g to remove the lipid layer.

**Preparation of reagents**
- **PBS-Tween Buffer:** Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 ml per plate by adding 25 ml PBST solution to 475 ml distilled water and mix thoroughly. N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.
- **Anti-Bovine IgG Conjugate:** Reconstitute the lyophilized HRP Conjugate with 11.5 ml PBS-Tween Buffer. Add the buffer carefully to the bottle. Leave the solution one minute and mix thoroughly. Prepare immediately before use. The remaining reconstituted conjugate can be stored at -20°C and thawed and refrozen up to 3 times.

**Precautions**
1. Carefully read and follow all instructions.
2. Store the kit and all reagents at +2 to +8°C (35 to 45°F).
3. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction booklets from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expire.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipet tip for each sample.
10. Do not pipet by mouth.
11. Include positive and negative serum and/or milk controls on each plate.

**Procedure**
1. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
2. **Serum Samples**
   - A. Add 100 µl of PBS-Tween Buffer to each well that will be used for serum samples and serum controls.
   - B. Add 4 µl of Positive Control Serum (Reagent A) and 4 µl of Negative Control Serum (Reagent B) respectively, to selected wells coated with IBR antigen. For confirmation purposes it is recommended to run the control sera in duplicates.
   - C. Add 4 µl of serum sample to a selected well coated with IBR antigen. For confirmation purposes it is recommended to run the samples in duplicates.
3. **Milk Samples**
   - A. Add 100 µl of Positive Control Milk (Reagent C) and 100 µl of Negative Control Milk (Reagent D) respectively, to selected wells coated with IBR antigen. For confirmation purposes it is recommended to run the control sera in duplicates.
   - B. Add 100 µl of skim milk sample to a selected well coated with IBR antigen. For confirmation purposes it is recommended to run the samples in duplicates.
4. Shake the plate thoroughly. Seal the plate and incubate at 37°C (98.6°F) for 1 hour.
5. Rinse the plate 3 times with PBS-Tween Buffer: fill up the wells at each rinse, empty the plate and tap hard to remove all remains of fluid.
6. Add 100 µl of HRP Conjugate to each well and incubate at 37°C (98.6°F) for 1 hour.
7. Repeat step # 5
8. Add 100 µl Substrate Solution to each well and incubate for 10 minutes at room temperature. Begin timing after the first well is filled.
9. Stop the reaction by adding 50 µl of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #8.
10. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

**Interpretation of the results**

**Criteria for test validity**
To ensure validity the Positive Control Serum/Milk should have a mean OD value greater than 1.2 and the Negative Control Serum/Milk should have a mean OD value of less than 0.2. For invalid tests, technique may be suspect and the assay should be repeated.

**Calculation of Cut-off**
- **Serum:**
  \[
  A = \frac{OD_{\text{value negative control}}}{2.5} 
  \]
- **Milk:**
  \[
  A = \frac{OD_{\text{value negative control}}}{2.0} 
  \]
If \( A > 0.2 \): Use \( A \) as your Cut-off
If \( A < 0.2 \): Use 0.2 as your Cut-off

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**Recommendation!**

Reconstituted conjugate may not be stored in refrigerator.