Enzootic Bovine Leukosis Virus Ab ELISA Kit

Catalog Number KA4891
480 assays
Version: 01

Intended for research use only
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Introduction

Intended Use

Enzyme immunoassay for the detection of antibodies to Bovine leukaemia virus in individual and pooled bovine blood serum samples.
The kit is standardized according to international standards, including E05. The kit detects O.I.E standard serum named E05 in 1:100 dilution, allowing examination of ten pooled samples.

Background

Enzootic Bovine Leukosis Virus (EBLV) belongs to the family Retroviridae. Enzootic bovine leukosis (EBL) is an infectious disease of cattle. The virus infects primarily B lymphocytes and elicits a persistent antibody response against five viral proteins.
The strongest antibody response is elicited against the surface glycoprotein gp-51 and the inner protein p-24. Only about 11% of infected animals develop symptoms of persistent lymphocytosis and lymphosarcomatosis. Current diagnostic techniques are based on the detection of specific antibodies. The routinely used methods are agar gel precipitation test (AGPT) to determine serum antibodies, ELISA or RIA. Due to its high sensitivity and specificity, ELISA is used to detect antiviral antibodies in the sera evaluated as negative by AGPT. This technique meets the requirements for testing pooled samples of these materials with a sensitivity corresponding to the requirements of the O.I.E. (EU Directive 88/406).

Principle of the Assay

The kit is intended for detection of specific antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen - antibody from the analysed sample - labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to bovine immunoglobulin conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific antibodies in the sample.
Antigen Used: purified and inactivated BLV antigen containing p-24 (inner protein)
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtitre Plate: Coated with antigen, 12 x 8 wells in bag with desiccant.</td>
<td>96 wells x 5</td>
</tr>
<tr>
<td>Negative Control Serum: 10x concentrated bovine serum containing no specific antibodies.</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>Positive Control Serum - limit: 10x concentrated bovine serum containing specific antibodies.</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>Conjugate: 100x concentrated peroxidase labelled antibodies to bovine IgG.</td>
<td>0.8 mL</td>
</tr>
<tr>
<td>Sample Diluent 13: Buffer with protein stabilizers, ready to use.</td>
<td>240 mL</td>
</tr>
<tr>
<td>Conjugate Diluent 11: Buffer with conjugate stabilizers, ready to use.</td>
<td>70 mL</td>
</tr>
<tr>
<td>TMB-Complete 5: Chromogenic substrate solution containing TMB/H₂O₂, ready to use.</td>
<td>60 mL</td>
</tr>
<tr>
<td>Wash Solution: 20x concentrated buffer.</td>
<td>2 x 60 mL</td>
</tr>
<tr>
<td>Stop Solution: Acid solution, ready to use.</td>
<td>60 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid (the shelf life of the kit is 24 months from the date of manufacture). The opened kit should be used within three months.

Materials Required but Not Supplied

- Single and multichannel pipettes
- Disposable tips
- Microplate washer
- Timer
- Microplate shaker (not necessary when a smaller group of samples is tested)
- Incubator (37°C) with moist air chamber
- Microplate reader
Precautions for Use

✓ Safety Precautions
1. The kit is intended for in vitro diagnostic use only.
2. Sera, Conjugate, Sample Diluent, Conjugate Diluent and other materials in contact with tested samples need to be handled as potentially infectious.
3. Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.
4. Stop Solution contains diluted acid solution. Avoid contact with eyes and skin!
5. It is necessary to observe the local safety rules and regulations.
6. In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solution containing serum, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

● Remnants disposal
All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

● Expired kit disposal
Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

✓ Procedural Notes
1. In order to obtain reliable results, it is necessary to strictly follow the Instructions for Use. Always use clean preferably disposable tips and glassware.
2. Slides - in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.
3. Wash Solution: use high quality distilled water for preparing the working strength Wash Solution.
4. Washing procedure: keep to the prescribed number of washing cycles and fill the wells to the upper edge.
5. TMB-Complete: the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.
6. Non-reproducible results might be caused by improper methodology as following:
   ● Insufficient mixing of reagents and samples before use
   ● Improper replacement of vial caps
   ● Using the same tip for pipetting different reagents
   ● Reagent exposure to excessive temperature; bacterial or chemical contamination
   ● Insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- Contamination of the wells edges with the Conjugate or samples
- Using reagents from different kit lots
- Contact of reagents with oxidants, heavy metals and their salts

7. The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

8. The kit might be used in all types of automatic EIA analyzers.

9. The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.
Assay Protocol

Reagent Preparation

✓ Dilute the Wash Solution 1:20. E.g. 60 mL of the concentrated Wash Solution + 1140 mL of distilled water (for 1 microplate: 15 mL of the Wash Solution + 285 mL of distilled water).
Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

✓ The Sample Diluent is ready to use, do not dilute further.

✓ The Conjugate Diluent is ready to use, do not dilute further.
Dilute the Conjugate 1:101 with the Conjugate Diluent. Add the Conjugate Diluent to 600 μL of the Conjugate to get 60 mL of the final volume (for one microplate: 120 μL to get 12 mL, for one strip: 10 μL to get 1 mL). Dilute the Conjugate 10 minutes prior to use at the earliest. Mix well.

✓ TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further.

Sample Preparation

Mix gently the Sample Diluent prior to use.

✓ Sample Preparation and Storage
Blood serum can be used for testing. Samples can be stored at +2°C to +8°C for 48 hours. For a longer period, store samples at -20°C.

✓ Dilution of serum samples and Control Sera
Dilute well the mixed samples and the Control Sera (PCS-L a NCS) 1:10 with the Sample Diluent.
E.g.: 10 μL of the sample + 90 μL the Sample Diluent.
Dilute in microtitre plate wells (see 8 Assay Procedures). Mix well.
The diluted samples should be used as soon as possible.

Assay Procedure

✓ Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unused strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls and the diluted samples according to the Plate Layout.
   ● Pipette 100 μL of the Sample Diluent into A1 well (blank).
   ● Pipette 90 μL of the Sample Diluent into the other wells.
   ● Pipette 10 μL of the Negative Control Serum into 2 wells (B1, C1).
• Pipette 10 μL of the Positive Control Serum - limit into 2 wells (D1, E1).
• Pipette 10 μL of the tested samples into the other wells with the Sample Diluent (F1 - H12).
• Mix thoroughly (using a microtitre plate shaker).

2. Cover the microplate with the lid and incubate at 37°C for 60 minutes or +2°C to +8°C in a moist air chamber overnight (14-18 hours). Before aspiration, incubate the microplate at room temperature for 15 minutes.

3. Aspirate the content of the wells and wash 4x with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.

4. Pipette 100 μL of the working strength Conjugate into all wells.

5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.

6. Aspirate the content of the wells and wash 4x with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.

7. Pipette 100 μL of TMB-Complete into all wells. Avoid contamination (see Procedural Notes section).

8. Cover the microplate with the lid and incubate at room temperature for 15 minutes.

9. Stop the reaction by adding 100 μL of the Stop Solution in the same order and intervals as the substrate was added.

10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

Observe blue colour development especially in wells with Positive Control Serum - limit (PCS-L). In case of a weaker reaction caused e.g. by lower room temperature, extend the incubation with substrate up to 30 minutes. Stop the reaction when the colour intensity of PCS-L corresponds to absorbance value 0.500 - 2.500.
Data Analysis

Calculation of Results

✓ Quality Control
  • The test is valid if:
    The absorbance of blank is lower than 0.200.
    \[
    \text{BLANK} < 0.150
    \]
    The absorbance of the Negative Control Serum is lower than 1/3-fold of the absorbance of the Positive Control Serum - limit.
    \[
    \text{Negative Control Serum} < \frac{1}{3} \times \text{Positive Control Serum} - \text{limit.}
    \]
    The absorbance of the Positive Control Serum - limit is within a range of 0.500 - 2.500.
    \[
    0.500 < \text{Positive Control Serum} - \text{limit.} < 2.500
    \]
    If the above specifications are not fulfilled, the test is not valid and must be repeated.

✓ Results Interpretation
  • Calculation of S/P ratio
    Divide the absorbance of the tested sample by the mean absorbance of the Positive Control Serum - limit (PCS-L) measured in the same test run:
    \[
    \frac{\text{Absorbance of sample}}{\text{Mean absorbance of PCS-L}} \times 100\% 
    \]
    Interpretation of test results is described in Table 1 and.

<table>
<thead>
<tr>
<th>S/P [%]</th>
<th>Evaluation</th>
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</thead>
<tbody>
<tr>
<td>lower than 30</td>
<td>negative</td>
</tr>
<tr>
<td>30 to 40</td>
<td>borderline</td>
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<tr>
<td>higher than 40</td>
<td>positive</td>
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</tbody>
</table>

Examination should be repeated in case of borderline results. Collect and test a new sample.

Table 2 Interpretation of test results of individual samples

<table>
<thead>
<tr>
<th>S/P [%]</th>
<th>Evaluation</th>
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</thead>
<tbody>
<tr>
<td>lower than 40</td>
<td>negative</td>
</tr>
<tr>
<td>higher than 40</td>
<td>positive</td>
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</tbody>
</table>

Borderline result can´t be used for individual samples.
### Resources

### Plate Layout

### Working Schedule

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>Negative Control Serum</td>
<td>Negative Control Serum</td>
<td>Positive Control Serum - limit</td>
<td>Positive Control Serum - limit</td>
<td>Sample</td>
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