



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

Distributor:
bioScreen
European Veterinary Disease
Management Center GmbH
Bemeroder Str.31
D- 30559 Hannover
Deutschland

bioScreen Ileitis Antibody ELISA

KIT FOR THE DEMONSTRATION OF SPECIFIC ANTIBODIES AGAINST *Lawsonia intracellularis* IN PORCINE SERA AND PLASMAS.

BLOCKING IMMUNOENZYMATIC TECHNIQUE

192 single well reactions

I. PRINCIPLE OF THE TEST

The bioScreen Ileitis Antibody ELISA kit uses an immunoenzymatic technique allowing the detection of anti-*Lawsonia intracellularis* antibodies in porcine serum and plasma samples.

The reaction is composed of three steps:

1. Each serum or plasma sample is placed in a well sensitised with the *Lawsonia intracellularis* Ag. Antibodies present in the sample bind to the bacterial antigen coated at the bottom of the well.

2. After a wash step, an anti-*Lawsonia intracellularis* monoclonal antibody (Mab) peroxidase conjugate is added. This monoclonal antibody, which binds to a *Lawsonia intracellularis* specific epitope, fixes onto the free antigenic sites forming a complex:
(Ag) - (Mab anti-protein / peroxidase).

3. Excess conjugate is eliminated by a second 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 :

- 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-*Lawsonia intracellularis* 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 12 strips of 8 wells sensitised with <i>Lawsonia intracellularis</i>	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate: <i>L. intracellularis</i> specific MAb - peroxidase (CJ) (100 X concentrated)	Dilute 100 times in the conjugate diluent and use within 2 h after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (NC)	Dilute as the samples
Positive control (PC)	Dilute as the samples
Sample diluent (SD)	Ready-to-use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Conjugate diluent (CD)	Ready-to-use.
Stop solution (S) Attention caustic!	Ready-to-use.
Adhesive films	6 films

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

Reference : ASIL1.NA version n2 – 11/04/2012

The chapters modified since the last version are in italic type. Version n1 → n2: Updated contact details of bioScreen.

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III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 10 to 1000 µl. Measurement deviation should be lower than 5% for every distributed volume.
- 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
- Incubator (+37 ± 3°C)

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:

R23/25: Toxic by inhalation and if swallowed.

R35 : Causes severe burns.

R36/37/38: Irritating to eyes, respiratory system and skin.

R41: Risk of serious damage to eyes.

R42/43: May cause sensitization by inhalation and skin contact.

S7: Keep container tightly closed.

S24: Avoid contact with skin.

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.

V. SAMPLES

The reaction is performed on individual sera and plasma diluted at **1:10**. 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 and controls to be tested.

For individual samples:

Prepare at least 100µl of sample diluted at 1:10 in sample diluent.

For controls:

After shaking the vials, prepare at least 200µl of each control diluted at 1:10 in sample diluent.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

Dispense 100 µl of previously diluted negative control (NC) to wells A1 and A2, and positive control (PC) to wells A3 and A4.

For a direct dilution in test well, dispense 90 µl of sample diluent and 10 µl of the controls.

2. Sample distribution:

Distribute 100 µl of previously diluted samples per well. The samples can be tested single or in duplicate.

For a direct dilution in test well, dispense 90 µl of sample diluent and 10 µl of serum or plasma.

- 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.

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

3. Incubation of the plate:

- Incubation protocol: 1 hour (± 5 min) at +37 ± 3°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 3 times.

Sufficient and homogenous washing of the plate is absolute crucial to afford valid results. The use of an automated plate washer or a wash bottle with spout tube is highly recommended. The used washing pressure should not exceed 0,2 bar.

II - ADDITION OF CONJUGATE

1. Preparation of conjugate:

Dilute the concentrated conjugate (CJ) 1:100 in the conjugate diluent (CD). 1 ml is needed for one strip, meaning 10 µl of CJ in 990 µl of CD.

2. Distribution of conjugate:

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

3. Incubation of conjugate:

Incubate 1 hour (± 5 min) at +37 ± 3°C.

WASHING:

Carefully remove the adhesive film, empty the wells and wash 3 times as described above.

III - REVELATION

1. Addition of the substrate:

Add 100 µl of buffered peroxidase 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 homogenisation.

2. Incubation of substrate:

10 ± 2 min at laboratory temperature (+20 ± 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 bubbles occur 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

The results of each test run are valid if:

- the OD of the negative controls (NC) is ≥ 0.500, and,
- the percentages of inhibition (PI value) of the positive controls (PC) are ≥ 40 %.

This percentage is calculated as follows:

$$PI = \frac{\overline{OD NC} - \overline{OD PC}}{\overline{OD NC}} \times 100$$

OD = average of the optical densities of an individual sample or control.

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

CALCULATION OF THE PERCENTAGE OF INHIBITION (PI)

For each sample:

$$PI = \frac{\overline{OD NC} - \overline{OD Sample}}{\overline{OD NC}} \times 100$$

OD = average of the optical densities, if the test is performed in duplicate.

- Any serum or plasma sample presenting a percentage of inhibition (PI) ≥ 30 % is considered **positive**.

- Any serum or plasma sample presenting a percentage of inhibition (PI) < 30 % is considered **negative**.

Within the negative range of results any sample presenting a PI-value between 20 and 30 should be considered questionable. A second test on a different sample from the same animal is recommended.

Result interpretation:

		PI 20	PI 30	
Serum or plasma	-	-	+	PI -value
		questionable range		

bioScreen
European Veterinary Disease
Management Center GmbH
Bemeroder Str. 31
D- 30559 Hannover
Germany
Tel: +49 511 89963960
Fax: +49 511 89963961
diagnostics.support@bioscreen-ms.de

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