**Legipid® Legionella Fast Detection**

Catalog number:

311-10-01

**Leaflet**

Test for rapid detection of *Legionella spp* in water samples, based on the combination of immunomagnetic capture and enzyme immunoassay (CEIA).
I. INTRODUCTION

II. TECHNOLOGY BEHIND THE TEST Legipid® Legionella Fast Detection

III. KIT REAGENTS AND COMPONENTS

IV. EXPIRY AND STORAGE

V. MATERIAL REQUIRED BUT NOT PROVIDED

VI. CAUTIONS AND RECOMMENDATIONS

VII. PROTOCOL

A. SAMPLE PREPARATION

B. ANALYSIS

C. RESULTS AND INTERPRETATION OF TEST

VIII. CONFIRMATION OF POSITIVE RESULT

IX. FEATURES AND VALIDATION OF TEST

X. REFERENCES
I. INTRODUCTION

Legipid® Legionella Fast Detection (Cat. No. 311-10) It is a simple and quick test for presumptive detection of Legionella spp in tap, natural and industrial water. The test combines magnetic immunocapture and enzyme-linked immunoassay (CEIA) with enzymatic colorimetric reaction for quick 1 hour test, after pre-concentrate a sample.

II. TECHNOLOGY BEHIND THE TEST Legipid® Legionella Fast Detection

The original sample of water is concentrated by filtration or the like, and this prepared sample is eluted and dispensed into a cuvette to be analyzed by the method CEIA. A suspension of magnetic particles that bind to Legionella is added. If Legionella cells are present in the prepared sample, they will bind to the antibodies immobilized on magnetic particles to form complex bacteria / particle. As these complexes may be separated by a magnet, they are easily washed and resuspended. The complexes are incubated with an anti-Legionella antibody conjugated with an enzyme, to form labeled complexes. After washing the Legionella / particle complexes are visualized colorimetrically, when the enzyme substrates are added. This test includes the following 3 main stages:

III. KIT REAGENTS AND COMPONENTS

The reference 311-10-01 (10 tests) contains the elements listed in the following table:

<table>
<thead>
<tr>
<th>Reagent/component</th>
<th>ID</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent</td>
<td>L0</td>
<td>1 flask (110 mL)</td>
</tr>
<tr>
<td>Capture reagent (immunomagnetic particles)</td>
<td>L1</td>
<td>10 single-doses (10 x 1 mL)</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>L2</td>
<td>1 flask (200 mL)</td>
</tr>
<tr>
<td>Enzyme conjugated Anti-Legionella antibody</td>
<td>L3</td>
<td>10 single-doses (10 x 1 mL)</td>
</tr>
<tr>
<td>Enzymatic cosubstrates</td>
<td>L4</td>
<td>5 tetra doses (5 x 5mL)</td>
</tr>
<tr>
<td>Stop reagent</td>
<td>L5</td>
<td>1 flask (2 mL)</td>
</tr>
<tr>
<td>Cuvette</td>
<td>CB</td>
<td>10 units</td>
</tr>
<tr>
<td>Disposable pipette</td>
<td>DP</td>
<td>15 units</td>
</tr>
</tbody>
</table>

The concentrator MP4-Hunter (311-MP4-SP) contains the elements listed in the following table:

<table>
<thead>
<tr>
<th>MP4-Hunter (ref. 311-MP4-SP), unit</th>
<th>Component</th>
<th>ID</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertable holder for 4 cuvettes</td>
<td>311-MP4-CH</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Magnetic holder for 4 cuvettes</td>
<td>311-MP4-MH</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clamping support</td>
<td>311-MP4-BB</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

The positioning mat (311-MP4-TC) avoids excessive proximity between the magnets. If not available, maintain a distance of at least 12 cm between concentrators.

The assembly and disassembly of the concentrator is as follows:
ASSEMBLY MP4

Insert cuvette into the holder (CH). Enter each cuvette holder (CH) in a magnetic holder (MH). Fit the unit in its clamping support (BB) (pressure slightly ahead and push it down).

DISASSEMBLY MP4

Disengage the clamping support (BB). Separate the cuvette holder (CH) of the magnetic holder (MH).

The holder with inserted cuvettes (CH) can be either manually shaked on the positioning mat (311-MP4-TC) or using an orbital shaker (311-MP4-AGT).
IV. SHELF LIFE AND STORAGE

Once received, the kit is stored between +2°C and +8°C, preferably at +4°C. The expiration of the reagents, properly stored, is 5 months from the date of manufacture. All reagents include their own batch number and storage conditions. These conditions are also found in the packaging. In addition, the protocol includes code, lot number and expiration date, ensuring the traceability of all reagents. You can ask the manufacturer for a certificate of analysis.

V. MATERIAL REQUIRED BUT NOT SUPPLIED

- Graduated bottle with screw cap, for elution of the filter.
- Glass fiber filter, 2.7μm pore diameter, for use as a pre-filter (*). Membrane filter, 0.4μm pore diameter in the case of polycarbonate filters (**).
- Container for residue.
- Filtration apparatus (**), to pre-concentrate water samples by membrane filtration.
- Optional: Primelab (ref. 911-10-PL), colorimeter S2B (ref. 511-10-COL) and cuvettes for spectrophotometer reading (ref. 511-10-04, box 100 units).
- Optional: vortex mixer or sonicator to elute the filter (the elution can be done manually).

(*) The use of a glass fiber filter of 2.7 micron pore size as prefilter for water samples is recommended only for filtering very dirty samples.

(**) Smaller pore diameter is possible but it can hamper the filtration.

(***) Note: Contact us for detailed information on equipment recommended by our technical department

VI. PRECAUTIONS AND RECOMMENDATIONS FOR BEST RESULTS

- This test should be performed by properly trained personnel.
- This test is designed for the following matrices: tap, natural and industrial water.
- The product is safe under normal conditions of use. Avoid contact with the eyes. If splashing might occur, wear safety glasses.
- Avoid skin contact by wearing gloves. (See MSDS)
- Attention: Certain isolates cannot be detected below 10⁶ CFU.
- Stable products. It is unlikely to react dangerously under normal conditions of use.
- The product should be disposed of in accordance with current regulations. Dispose of empty containers through the process of recycling or waste disposal.
- The assay performance depends on strict compliance with the following practices, especially regarding to the correct execution of the protocol:

- Do not use reagents after the expiration date.
- Using as negative control the same eluent (L0) used in sample preparation (elution).
- Use a negative control (L0 reagent) for each batch of test.
- Bring test reagents (18-26 ° C) at least 30 minutes before use.
- Shake the reagent L1 before use, to the homogeneity of the magnetic particles.
- Run carefully washing steps (reagent L2).
  - The cuvettes are disposable. Do not reuse.

- Leave to warm at least for 30 minutes the single-doses and tetra doses necessary to perform scheduled tests.
- Use the positioning mat (311-MP4-TC). If not, keep a distance among concentrators of at least 12 cm.
- For highly colored samples: after emptying supernatants, the assembled MP4 module can be tilted forward up to 45 °to allow the residual liquid to drain to the bottom of the cuvettes, in the opposite side of the retained magnetic beads. This allows the elimination of this liquid by using a pipette.
- The reagents are supplied in excess. Do not use of any reagent excess. Do not mix batches.
VII. PROTOCOL

It is strongly recommended to read this protocol carefully before starting the test.

A. Sample preparation

1. Collect the volume of the original water sample to be concentrated (e.g. by filtration).

2. Add 10 ml of eluent in a flask. Use L0 as eluent.

3. Filter the collected volume using a membrane filter (*). For very dirty samples you can use a glass fiber pre-filter of 2.7 µm pore diameter placed on top of the membrane filter.
   (*) AOAC PTM validation used 0.4 µm

4. Carefully separate the filter from the filtration system and put it into the flask with the eluent prepared in step 2. Optionally filter can be cut into pieces with scissors. If you have also used the pre filter, please remove it and discard.

5. Elute the filter by shaking. This agitation can be:
   a. Manual (2 minutes)
   b. Vortex (2 minutes)
   c. Ultrasonic bath (5 minutes)

Note:
For each sample batch, a negative control is performed with the same used eluent (L0)

Protocol based on the contents of the ISO 11731 standard for detection and enumeration of Legionella in water.

The eluted sample is called the prepared sample

For an optimal recovery, shake this sample just before transferring to the cuvette.
B. Analysis with the kit Legipid® Legionella Fast Detection

Before starting the test:

- Prepare only the mono-doses and tetra-doses needed and let 30 minutes at room temperature before use.
- Insert the cuvettes (CB) in its support (CH), as many as trials are going to be performed.

B.1) CAPTURE STAGE

1. Gently shake the L1 by repeatedly inverting the single-dose until a completely homogeneous suspension, and then add it completely in each cuvette.

![Image of cuvettes and L1](image1)

2. Add L0 to the control cuvette (C) up to line 3 (9ml) - just one control per testing batch -. Add the sample (previously filtered and eluted) in the test cuvette (T) up to line 3 (9ml), being careful not to let fall any pieces of filter.

![Image of L0 and sample addition](image2)

3. PLACE THE PLUGS into cuvettes. Shake the cuvette holder (CH) in gentle sway on the mat 3 times every 3 minutes, for 15 minutes, so that the cuvettes are horizontal.

![Image of plugs placement and shaking](image3)

4. REMOVE AND DISPOSE THE PLUGS (place the cuvettes upright). Assemble MP4 (see p. 4) and place in its position on the mat (TC). Wait 5 minutes to retain the magnetic particles

5. Discard supernatant by emptying the cuvette on the opposite side to magnets, by tipping this assembly up.

6. Disassemble the MP4 and put the cuvette holder (CH) upright on the mat. Then add the reagent L2 up to line 2 (4.5 ml) in each cuvette.

7. Shake WITHOUT PLUGS on the mat in vigorous shake swing to resuspend the particles for 10 seconds.

8. Assemble the MP4 and place it in its position on the mat (TC) and wait 3 minutes to retain the magnetic particles.

9. Discard supernatant emptying the cuvettes on the opposite side to the magnets, by tipping this assembly up.
B.2) MARKING STAGE

1. Disassemble the MP4 and put the cuvette holder (CH) on the mat (TC). Add a single-dose of reagent L3 (1 ml) in each cuvette.

2. Shake on the mat in vigorous shake swing WITHOUT PLUGS for 10 seconds and then gentle sway every 2 minutes for 10 minutes.

3. Assemble the MP4. Place it in its position on the mat (TC) and wait 3 minutes to retain the magnetic particles.

4. Discard supernatant by pouring on the opposite side to the magnets, by tipping up the assembly.

5. Disassemble the MP4 and put the cuvette holder (CH) on the mat (TC). Add the reagent L2 up to line 2 (4.5 ml) in each cuvette.

6. Shake for 10 seconds WITHOUT LIDS in energetic swing on the mat (TC) to resuspended the particles.

7. Assemble the MP4 and place it in its position on the mat (TC) and wait 3 minutes to retain the magnetic particles.

8. Repeat steps 4, 5, 6 and 7 (of this section B.2 MARKING STAGE) twice.

B.3) DETECTION STAGE

1. Discard supernatant by pouring on the opposite side to the magnets, by tipping up the assembly. Disassemble the MP4 and put the cuvette holder (CH) upright on the mat (TC).

2. Prepare the reagent L4 (one vial for each 4 trials): unseal the protective cap and remove it. Press the piston to be fully inserted into the shutter. Shake the mixture vigorously for 10 seconds. Once the reagent L4 is prepared, it should be used immediately.

3. Open the vial of L4 just before use. To do this remove the shutter with the piston inserted, applying light pressure with the thumb side.

4. Immediately add the L4 up to line 1 (1 ml) to each cuvette using disposable pipette (DP). Shake on the mat (TC) in gentle sway for 2 minutes. Start shaking in energetic swing for the first 10 seconds to resuspend the particles. (Note: count time from the first addition of L4)

5. Stop shaking. Add 3 drops of L5 (100 µl) to each and every one of the cuvettes and shake in the mat (TC) in gentle sway for 5 seconds. (Note: add reagent L5 in the same order as the L4 was added)

6. Assemble the MP4 and place it in their position on the mat (TC) and wait 5 minutes to retain the magnetic particles.
C. INTERPRETATION AND TEST RESULT REPORT

C.1. Visual Interpretation

The visual interpretation of the test results is summarized in the following diagram:

```
STOP after 2 minutes

NO

T > C

YES

NEGATIVE

POSITIVE/GO TO COLOR CHART

T: COLOR OF THE TEST  C: COLOR OF THE CONTROL
```

The result of the test (T) is considered **POSITIVE** if the test (T) presents more color than the control (C) after 2 minutes from the start of the colorimetric reaction. The overall estimate of the level of *Legionella* spp. can be obtained by comparing the color of the test (T) with the color chart.

<table>
<thead>
<tr>
<th>Color Chart</th>
<th>Position the cuvette test (T) with the following color chart.</th>
<th>Results as CFU equivalent / volume examined</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Color Chart Image" /></td>
<td>Below the first color of the card: up to two orders of magnitude (10^2 CFU_{eq} / volume examined). Below the second color of the card: up to three orders of magnitude (10^3 CFU_{eq} / volume examined). Over the second chart color: equal to or greater than four orders of magnitude (10^4 CFU_{eq} / volume examined).</td>
<td></td>
</tr>
</tbody>
</table>

The result of the test (T) is considered **NEGATIVE** if the test (T) has no color difference with the control (C) after 2 minutes since the beginning of the colorimetric reaction.

C.2. Optical reading

(1) Transfer 1ml of the supernatants Control (C) and Tests (T) each in its corresponding cell reading. *Important note: Pipette 1ml of the supernatant from the opposite side to the magnet, being careful not to drag the particles retained by the magnet.*

(If you have a Primelab device, follow the instructions; If you are using another colorimeter, continue with the following steps)

(2) Measure absorbance at 429 nm in a cell filled with distilled water. Set the absorbance at zero.

(3) Measure absorbance at 429 nm of the supernatant of the control (C), as reference. Readjust the absorbance to zero.

(4) Measure the absorbance of the supernatant of each test (T). Immediately read: always within 10 minutes since the end of the colorimetric reaction.

*Note: If the length of the light path is not 1 cm, the corresponding correction is necessary. Follow the manufacturer's instructions of your optical reader.*
Negative results - tests of samples with relative absorbance readings below the cutoff value (Ar = 0.04 units) are negative and are reported as Not Detected.

Positive results - tests of samples with relative absorbance readings above the cutoff value (Ar = 0.04 units) are positive and are reported as Detected.

(5) For positive results, obtain the log₁₀ of relative absorbance.

(6) Estimate the concentration of Legionella spp in the volume examined entering the value of the log₁₀ of the relative absorbance (Ar) in the following formula:

\[ y = 2.3061 \times 4.9815, \text{ where } x = \log_{10}(Ar) \text{ and } y = \log_{10}(\text{CFU}_\text{eq} / \text{volume examined}) \]

(7) The result is obtained by applying the inverse transformation of the logarithm:

\[ \text{Contamination (CFU}_\text{eq} / \text{volume examined}) = 10^y \]

Note: If desired, you can request an Excel spreadsheet programmed for automatic calculation of concentration.

At the end of the trial empty cuvettes and dispose. Do not reuse the cuvettes or the remains of reagents.

VIII. CONFIRMATION OF POSITIVE RESULTS

In the AOAC-RI certification, a positive result for Legipid® Legionella Fast Detection was considered a presumptive positive and was confirmed with standardized culture methods (for example ISO 11731: 1998) on a volume of 0.1-0.5 ml of the sample prepared. In the case of results that are inconsistent between Legipid® Legionella Detection Fast and the confirmation method, you should take steps to ensure the validity of results.

The positive deviation may be associated with poor recovery of target bacteria by culture (viable but not culturable cells -VBNC-) microbiota which inhibits growth of Legionella, etc., or insufficient compliance in protocolated washings steps in the labeling stage.

IX. CHARACTERISTICS AND VALIDATION OF TEST

Legipid® Legionella Fast Detection kit is a quick and simple test for the detection of Legionella spp in water samples. The relative level of detection is 93 CFU_eq / volume examined (LOD50). With optical reading the limit of detection is 40 CFU_eq / volume examined and the quantification limit is 60 CFU_eq / volume examined.

Legipid® Legionella Fast Detection is validated by the AOAC-Research Institute in the Performance Tested Method Program to potable, natural and industrial water. Certificate No. 111101

X. REFERENCES


User warning: Use this product only for environmental analysis

<table>
<thead>
<tr>
<th>Lot No.:</th>
<th>For Technical assistance contact: Biótica, Bioquímica Analítica, S.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parque Científico y Tecnológico, Universidad Jaume I</td>
</tr>
<tr>
<td></td>
<td>Edif. Espaitec 2, ground floor, lab 2</td>
</tr>
<tr>
<td></td>
<td>12071 – Castellón, Spain</td>
</tr>
<tr>
<td></td>
<td><a href="http://www.biottica.es">www.biottica.es</a> <a href="mailto:info@biottica.es">info@biottica.es</a></td>
</tr>
<tr>
<td></td>
<td>Tel.: +34 964108131 Fax: +34 964737790</td>
</tr>
</tbody>
</table>
### FAST GUIDE TEST LEGIPID 
**LEGIONELLA FAST DETECTION - PACK 10 - SCOPE QUALITATIVE AND QUANTITATIVE**

#### Filtration

<table>
<thead>
<tr>
<th>Component / Reagent</th>
<th>Vol (ml)</th>
<th>Units</th>
<th>Handling characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>— 1</td>
<td></td>
<td>Placing in the vacuum system</td>
</tr>
<tr>
<td>PF</td>
<td>— 1</td>
<td></td>
<td>Depositing PF on the FM</td>
</tr>
<tr>
<td>M.O. 1000</td>
<td>— 1000</td>
<td></td>
<td>Actuate the vacuum system to filter</td>
</tr>
<tr>
<td>L0</td>
<td>10</td>
<td></td>
<td>Annotate vol. really filtering</td>
</tr>
<tr>
<td>M.P. 9</td>
<td>— 9</td>
<td></td>
<td>Analyze as soon as possible (or reserve ≤ 24 hours at 2-8 °C)</td>
</tr>
</tbody>
</table>

#### Analysis

C = negative control; T = test; CH = cuvette holder; M.P. = prepared sample (eluate)

**Step 1 Capture - Insert the cuvettes in the cuvette holder (CH)**

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>Reagent</th>
<th>Line</th>
<th>ROCKING AGITATION</th>
<th>MAGNET</th>
<th>HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, T</td>
<td>L1</td>
<td>1</td>
<td>15 min suave</td>
<td>5 min</td>
<td>EMPTYING</td>
</tr>
<tr>
<td>C</td>
<td>L0</td>
<td>3</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
<tr>
<td>T</td>
<td>M.P.</td>
<td>3</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
</tbody>
</table>

**Step 2 Washing I - Add L2 on magnetic particles**

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>Reagent</th>
<th>Line</th>
<th>ROCKING AGITATION</th>
<th>MAGNET</th>
<th>HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, T</td>
<td>L2</td>
<td>2</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
<tr>
<td>C, T</td>
<td>L2</td>
<td>2</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
</tbody>
</table>

**Step 3 Labelling- Add L3 on magnetic particles**

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>Reagent</th>
<th>Line</th>
<th>ROCKING AGITATION</th>
<th>MAGNET</th>
<th>HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, T</td>
<td>L3</td>
<td>1</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
<tr>
<td>C, T</td>
<td>L3</td>
<td>1</td>
<td>10 min</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
</tbody>
</table>

**Step 4 Washing II - 3 TIMES - Add L2 on magnetic particles**

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>Reagent</th>
<th>Line</th>
<th>ROCKING AGITATION</th>
<th>MAGNET</th>
<th>HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, T</td>
<td>L2</td>
<td>2</td>
<td>10 sec</td>
<td>3 min</td>
<td>EMPTYING</td>
</tr>
</tbody>
</table>

**Step 5 Detection**

DP: disposable pipette

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>Reagent</th>
<th>Line</th>
<th>ROCKING AGITATION</th>
<th>MAGNET</th>
<th>HANDLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, T</td>
<td>L4</td>
<td>1 ml (DP)</td>
<td>10 sec</td>
<td>10 sec</td>
<td>MIX L4 AND USE</td>
</tr>
<tr>
<td>C, T</td>
<td>L5</td>
<td>3 drops</td>
<td>5 sec</td>
<td>5 min</td>
<td>PUT SUPERNATANT INTO A SEMI-MICRO CUVETTE (QUANTITATIVE) or go to COLOR CHART (QUALITATIVE)</td>
</tr>
</tbody>
</table>

**Filtration**

FM = Membrane Filter; PF = Prefilter; M.O. = Original Sample; M.P. = Prepared Sample

**Handling characteristics**

- Placing in the vacuum system
- Depositing PF on the FM
- Actuate the vacuum system to filter
- Annotate vol. really filtering
- Eluir FM (descartar el PF), SHAKING options:
  - Manual, 2 min
  - Vortex, 2 min
  - Sonication, 5 min

**Analysis**

- CH HORIZONTAL ON MAT, WITH PLUGS
- MP4 ON MAT, WITHOUT PLUGS
- MP4 ON MAT, WITHOUT PLUGS