Listeria monocytogenes p60 ELISA Kit

Listeria monocytogenes p60 ELISA Kit is an Enzyme Immunoassay kit for the quantification of Listeria monocytogenes p60 in food samples.

Catalog number: ARG83363

Package: 96 wells

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INTRODUCTION

Listeria monocytogenes is the species of pathogenic bacteria that causes the infection listeriosis. It is a facultative anaerobic bacterium, capable of surviving in the presence or absence of oxygen. It can grow and reproduce inside the host's cells and is one of the most virulent foodborne pathogens: 20 to 30% of foodborne listeriosis infections in high-risk individuals may be fatal.

Listeria monocytogenes is a Gram-positive bacterium, in the phylum Bacillota. Its ability to grow at temperatures as low as 0 °C permits multiplication at typical refrigeration temperatures, greatly increasing its ability to evade control in human foodstuffs. Motile via flagella at 30 °C and below, but usually not at 37 °C. L. monocytogenes can instead move within eukaryotic cells by explosive polymerization of actin filaments (known as comet tails or actin rockets). Once Listeria monocytogenes enters the host cytoplasm, multiple changes in bacterial metabolism and gene expression help to complete the metamorphosis of its from soil dweller to intracellular pathogen.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Listeria monocytogenes p60 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Listeria monocytogenes p60 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Listeria monocytogenes p60 is added to each well and incubate.

Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Listeria monocytogenes p60 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Listeria monocytogenes p60 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
Standard	1 vial (100 µL; 4 µg/mL)	-20°C
Diluent Buffer	50 mL	4°C
1000X Listeria monocytogenes p60 Antibody	1 vial (20 µL)	4°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is

blue before use, DO NOT USE IT.

- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Food- Collection samples, transfer 1 mL of the culture to a microcentrifuge tube and inactivate the bacteria by heating the sample at 90-100°C for 30 min. After cool down to room temperature, culture supernatant is collected by centrifugation at 10,000 rpm for 5 min.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into 1X distilled water to yield 1X Wash Buffer (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL).
- 1X Listeria monocytogenes p60 Antibody: 10 minutes before use, dilute 1000X Listeria monocytogenes p60 Antibody into Diluent Buffer to yield 1X Antibody Conjugate.
- 1X HRP-Streptavidin Solution: 10 minutes before use, dilute 1000X HRP-Streptavidin Solution into Diluent Buffer to yield 1X HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- Standard: Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	Listeria monocytogenes p60 (ng/mL)	Diluent Buffer (µL)	Standard (µL)
S1	40	990	10 (4 µg/mL Stock)
S2	20	500	500 of S1
S3	10	500	500 of S2
S4	5	500	500 of S3
S5	2.5	500	500 of S4
S6	1.25	500	500 of S5
S7	0.625	500	500 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

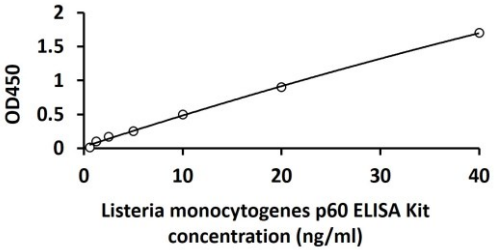
1. Add **100 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **2 hour** at **37°C**.
3. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Listeria monocytogenes p60 Antibody** to each well.
5. Cover the plate and incubate for **1 hour** at **room temperature**.
6. Aspirate each well and **wash** plate **as step 3**.
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
8. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
9. Aspirate each well and **wash** plate **as step 3**.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **5-30 mins** at **room temperature** in the dark.
12. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Listeria monocytogenes p60 ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

25 ng/ml

Assay Range

0.625 - 40 ng/ml