Product Manual

Listeria monocytogenes p60 ELISA Kit

Catalog Numbers

BCT-5176 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Listeriosis is a serious infection caused by the germ Listeria monocytogenes. People usually become ill with listeriosis after eating contaminated food. The disease primarily affects pregnant women, newborns, older adults, and people with weakened immune systems.

The initial internalization of Listeria monocytogenes in the mammalian cells occurs through the surface proteins internalin (InlA and InlB). However, the pore-forming protein listeriolysin O (LLO) is the primary virulence factor of Listeria monocytogenes, as it helps the bacteria to escape phagosome of host cells and further multiplication inside the host cytoplasm.

The p60 protein encoded by the *iap* (invasion-associated protein) gene is expressed on the cell surface, but it is mainly secreted in large quantities in the culture supernatant of Listeria monocytogenes. This makes the p60 protein an ideal indicator protein for the detection of Listeria monocytogenes from contaminated foods.

Cell Biolabs' Listeria monocytogenes p60 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of the Listeria monocytogenes p60 protein. The ELISA monoclonal antibodies specifically recognize the p60 protein secreted from Listeria monocytogenes and will not react with other food borne bacteria such as E. coli O157, Salmonella, and Shigella. The ELISA kit has a detection sensitivity limit of 625 pg/mL Listeria monocytogenes p60. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and food test samples.

Assay Principle

An anti-Listeria monocytogenes p60 monoclonal antibody is adsorbed onto a microtiter plate. Listeria monocytogenes p60 present in the pre-enriched sample or standard binds to the antibody adsorbed on the plate; an FITC-conjugated anti-Listeria monocytogenes p60 monoclonal antibody is added and binds to the Listeria monocytogenes p60 captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-Listeria monocytogenes p60 monoclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and Substrate Solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of Listeria monocytogenes p60 present in the sample. The reaction is terminated by addition of Stop Solution and the absorbance is measured at 450 nm. A standard curve is prepared from the provided recombinant Listeria monocytogenes p60 standard and the sample Listeria monocytogenes p60 amount is then determined.

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Listeria monocytogenes p60 Antibody Coated Plate (Part No. 51761B): One strip well 96-well plate.
2. FITC-Conjugated Anti-Listeria monocytogenes p60 Monoclonal Antibody (Part No. 51762C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.

5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant Listeria monocytogenes p60 Standard (Part No. 51763D): One 100 µL vial of 4 µg/mL E.coli expressed recombinant Listeria monocytogenes p60 (Met1-Val484) in PBS containing BSA.

Materials Not Supplied

1. Listeria monocytogenes sample: Food, Fecal or Environmental Samples
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store recombinant Listeria monocytogenes p60 Standard at -20 ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your Listeria monocytogenes samples contain potentially pathogenic organisms before heat inactivation; you must follow the recommended NIH and FDA guidelines for all materials containing pathogenic organisms.

Sample Preparation and Enrichment

Homogenize 25 g of samples (ground beef, milk, lettuce, etc) in 225 mL of Listeria monocytogenes culture medium, such as buffered Listeria enrichment broth (BLEB). Incubate for 4 hours at 30ºC, then add Selective supplements (10 µg/mL of acriflavin hydrochloride, 40 µg/mL of nalidixic acid and 50 µg/mL of cycloheximide). Continue to incubate at 30ºC for a total of 48 hrs.

Post Enrichment Heat Inactivation

After the enrichment incubation, transfer 1 mL of the culture to a microcentrifuge tube and inactivate the bacteria by heating the sample at 90-100ºC for 30 min. After cool down to room temperature, culture supernatant is collected by centrifugation at 10,000 rpm for 5 min and used in ELISA steps.

Note: The enriched non-inactivated sample should be kept until the ELISA results are obtained.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-Listeria monocytogenes p60 Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Recombinant Listeria monocytogenes p60 Standard in the concentration range of 40 ng/mL – 0.625 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	4 µg/mL Recombinant Listeria monocytogenes p60 Standard (µL)	Assay Diluent (µL)	Listeria monocytogenes p60 Standard (ng/mL)
1	10	990	40
2	500 of Tube #1	500	20
3	500 of Tube #2	500	10
4	500 of Tube #3	500	5
5	500 of Tube #4	500	2.5
6	500 of Tube #5	500	1.25
7	500 of Tube #6	500	0.625
8	0	500	0

Table 1. Preparation of Recombinant Listeria monocytogenes p60 Standard.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each Listeria monocytogenes p60 sample, standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of Listeria monocytogenes p60 sample or standard to the Anti-Listeria monocytogenes p60 Antibody Coated Plate.
4. Cover with a plate cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-Listeria monocytogenes p60 Monoclonal Antibody to each well.

7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Listeria monocytogenes p60 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

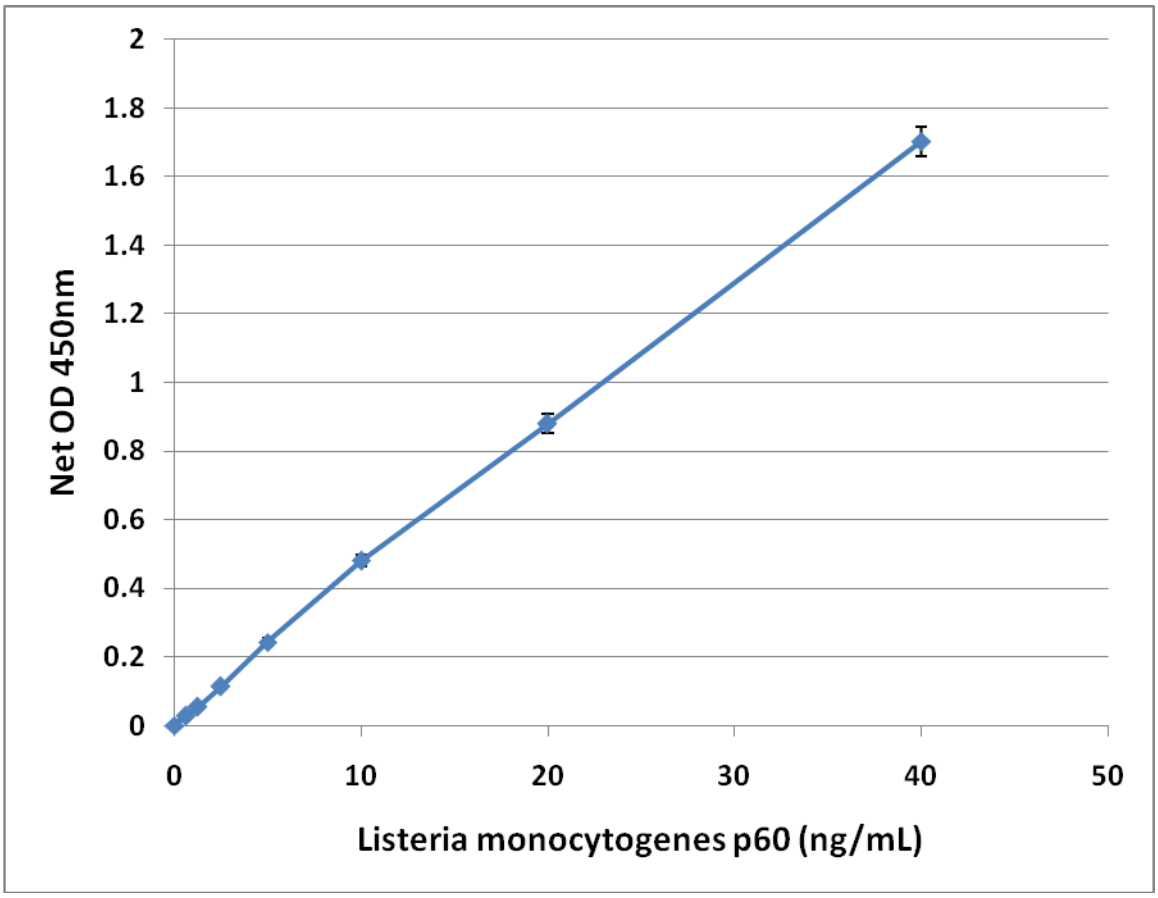

Figure 1: Listeria monocytogenes p60 Standard Curve.

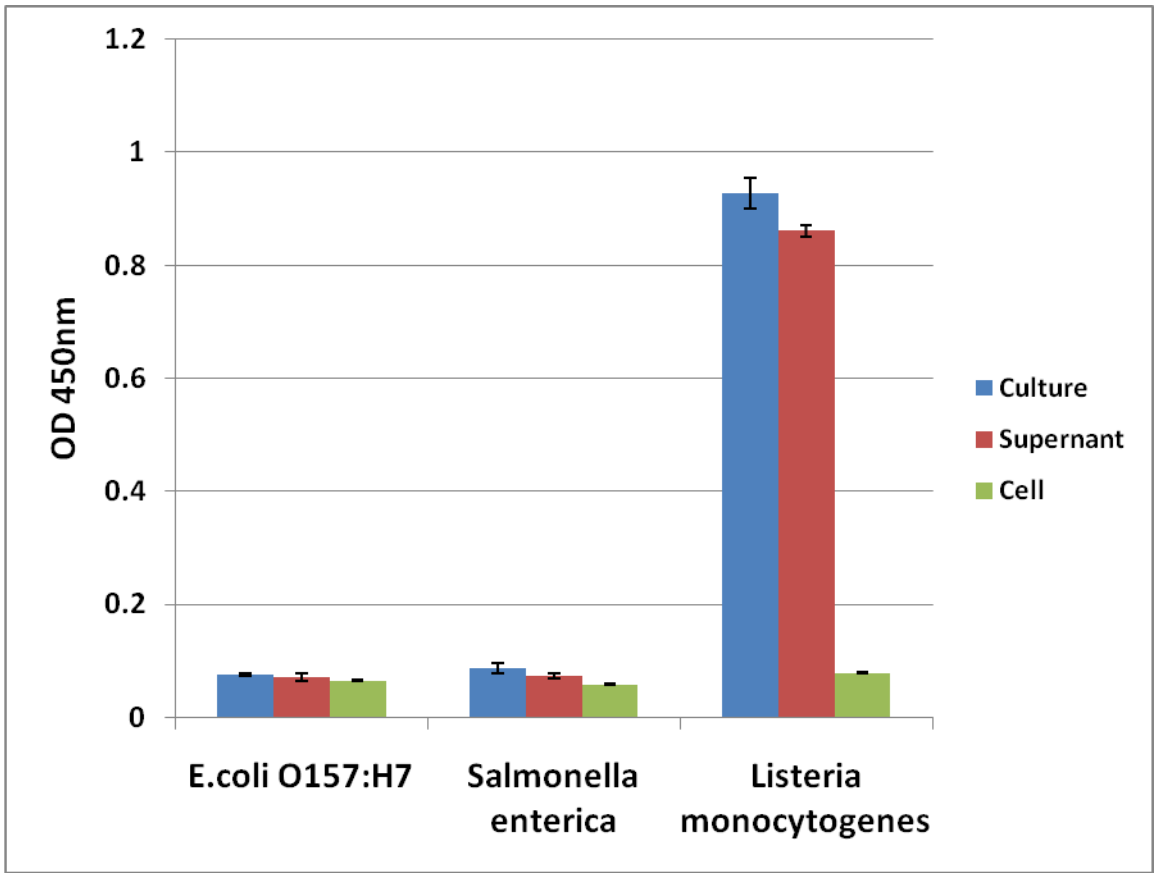

Figure 2: p60 is largely found the culture supernatant of Listeria monocytogenes. Heat inactivated overnight bacterial culture (Culture) is subjected to centrifugation to separate bacterial cells and culture supernatant (Supernatant). The cell pellet is resuspended with the same volume of culture medium (Cell). All bacterial samples are further diluted 100-fold in Assay Diluent and subjected to Listeria monocytogenes p60 ELISA Kit according to Assay Protocol.

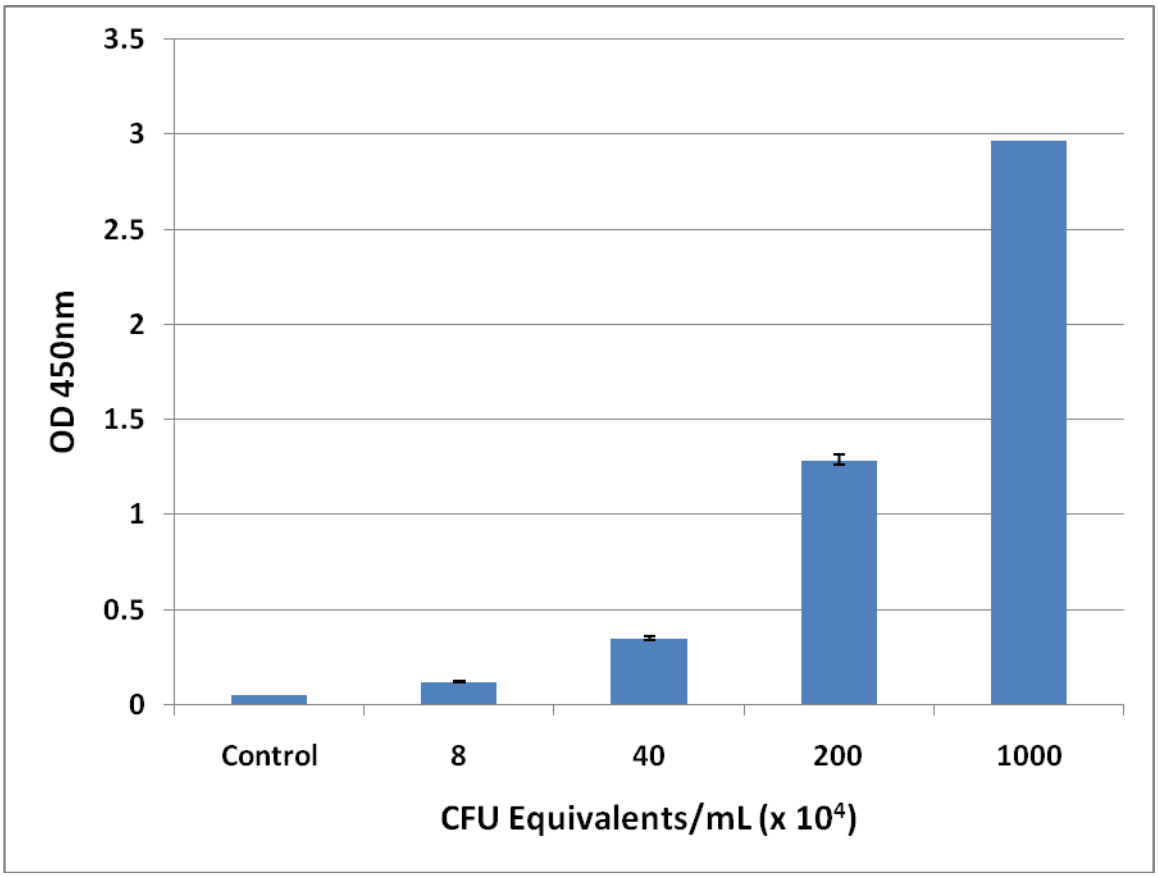

Figure 3: p60 in Listeria monocytogenes Culture Supernatant. 2.5 x 10^8 CFUs/mL of Listeria monocytogenes culture is first heat inactivated at 95ºC for 30 min. After centrifugation, cell culture supernatant is diluted in Assay Diluent and subjected to Listeria monocytogenes p60 ELISA Kit according to the Assay Protocol.

References

1. Schuchat A, Swaminathan B, Broome CV. (1991) Epidemiology of human listeriosis. *Clin. Microbiol. Rev.* **4**, 169–183.
2. Vázquez-Boland JA, Domínguez-Bernal G, González-Zorna B, Kreft J, Goebel W. (2001) Pathogenicity islands and virulence evolution in Listeria. *Microbes Infect.* **3**, 571–584.
3. Kuhn, M., and W. Goebel. (1989) Identification of an extracellular protein of *Listeria monocytogenes* possibly involved in intracellular uptake by mammalian cells. *Infect. Immun.* **57**, 55-61.
4. Bubert, A., M. Kuhn, W. Goebel, and S. Köhler. (1992) Structural and functional properties of the p60 proteins from different *Listeria* species. *J. Bacteriol.* **174**, 8166-8171.
5. Rowan NJ, et al. (2000) Virulent rough filaments of Listeria monocytogenes from clinical and food samples secreting wild-type levels of cell-free p60 protein. *J. Clin. Microbiol.* **38**, 2643–2648.

Warranty

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