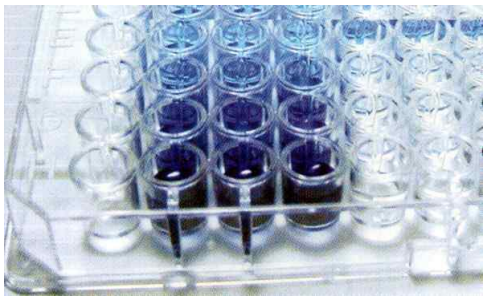
Listeria monocytogenes SURE *mono* ELISA Kit

Cat. No. AG-45A-0026EK-KI01



Instruction Manual

Issue Date 2009.09.01
Revised Date

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

AdipoGen™

Table of Contents

Introduction

The genus *Listeria* is comprised of six species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*. All *Listeria* species are widely found in nature as well as in many food industry-related applications (1). Among these species, *L. monocytogenes* is known to infect humans, causing sepsis, meningitis, or encephalitis (2). Due to the preferential infectivity of *L. monocytogenes* in infants, pregnant women, and immunocompromised patients and an associated high fatality rate, this organism can be classified as an important opportunistic infectious agent which is becoming an emerging problem in public hygiene (3, 4). In order to prevent *L. monocytogenes* infection, prescreening of processed foods with reliable diagnostics is necessary.

Assay Principles

This kit is enzyme-linked immunosorbent assay (ELISA) for qualitative determination of *L.monocytogenes*.

The SURE *mono* ELISA was originally described in following paper entitled “Use of Monoclonal Antibodies That Recognize p60 for identification of *Listeria monocytogenes*” *CDLI* 11(3): 446-451, 2004

Kit Components

1) Antibody coated 96-well plate, 12 X 8 well strips
2) 5x Wash concentrate, 100 ml
3) Positive control, 1 ug (lyophilized)
4) Diluent, 50 ml
5) Secondary antibody, 12 ml X 2
6) 100x Detector, 150 µl
7) Substrate Ⅰ, 6 ml
8) Substrate Ⅱ, 6 ml

Reagents Description

Antibody coated 96-well plate, 12x8 well strips, with an absorbed monoclonal antibody specific for *L. monocytogenes*
5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate
Positive control, lyophilized *L. monocytogenes* extract
Diluent, for reagent dilution
Secondary antibody, biotinylated polyclonal antibody against *L. monocytogenes*
100x detector, HRP conjugated streptavidin
Substrate Ⅰ and Ⅱ, chromogenic reagents

Storage of Reagents

Reagents must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solu tion may be stored at room temperature for up to one month.

Materials Required but not Supplied

Precision single and multi-channel pipettes.
Disposable pipette tips.
Microtubes or equivalent for preparing dilutions.
Disposable plastic containers for preparing working detector antibody and substrate.
Reagent reservoirs.
Microwell or microstrip plate reader 414 nm
Deionized water

Reagent Preparation

Before using the SURE *mono* ELISA kit preparation of reagents is necessary.
*CAUTION : Avoid cross-contamination of reagents. Ensure that caps for the reagent bottles are not inadvertently exchanged.

1X Wash Solution

Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 part deionized water). The diluted 1X Wash Solution is stable for one month at room temperature. Store the wash solution at 4°C when not in use. Ensure containers used for storing wash solution are thoroughly cleaned each time the are used. Where microbial contamination of water is a problem, the use of sterilized water is recommended.

Positive Control

Reconstitute with the addition of 1ml deionized water to the vial.

Negative Control

Diluent is used for negative control.

1X Detector

Dilute 100X Detector 1:100 with Diluent (1 part 100X Detector with 99 parts Diluent). Use the 1X Detector within one hour of preparation. Discard reconstituted conjugate after 1 month.

Substrate

Freshly prepare just before use the **substrate solution** by adding one part Substrate Ⅰ to one part Substrate Ⅱ.

Assay Procedure

Ensure all kit components are at room temperature (20-25°C) before use.

STEP 1 : Sample preparation
Transfer 1ml of the enrichment broth into each respective tube. Then, heat for 10 minutes in a boiling water bath. Cool the heated samples to room temperature.

STEP 2 : Preparation of sample wells
Open the pouch and remove the required number of wells from the sealing film, allowing one well for each sample, one for the positive control and one for the negative control. Press the wells firmly into place into place in the holder provided. Replace unused wells in the foil pouch and reseal strip provided.

STEP 3 : Sample addition
Using a new pipette tip for each sample, transfer 200$\mu\ell$ aliquots of the controls and samples into individual wells, recording the position of each sample on the plastic cling wrap film and incubate for 30 minutes at 35-37°C .

STEP 4 : First Washing
*Caution : Through washing of the wells is essential for drawing a clear interpretation of results.
Wash and completely empty the wells a total of 3 times with 250$\mu\ell$ 1X wash solution.

STEP 5 : Add 200$\mu\ell$ secondary antibody to each well.
Ensure the wells are empty before proceeding.
Incubate at 37°C for 30 minutes.

STEP 6 : Second Washing
Empty and wash them thoroughly a total of 3 times with 250$\mu\ell$ 1X wash solution.

STEP 7 : Add 200$\mu\ell$ Detector(1x) to each well.
Ensure the wells are empty before proceeding.
Incubate at 37°C for 30 minutes.

STEP 8 : Third washing
Empty and wash them thoroughly a total of **6 times** with 250$\mu\ell$ 1X wash solution.

STEP 9 : Add 100$\mu\ell$ Substrate to each well.
Ensure the wells are empty before proceeding.
Incubate at room temperature for 15 minutes.

STEP 10 : Read at 414 nm.

Continue the substrate incubation only until the Positive Control has reached an absorbance of 1.0 If the absorbance has not reached 1.0 after 15 minutes, the test result cannot be used. Refer to the trouble-shooting guide before repairing the test.

Performance Characteristics

A sample is considered NEGATIVE when the test procedure is valid and the sample well has a reading less than 0.5.

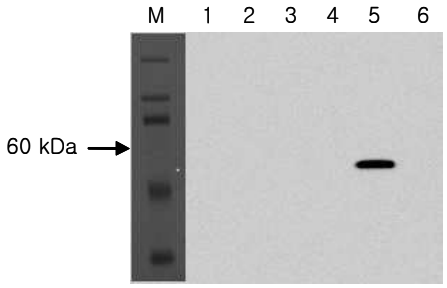
A sample is considered POSITIVE when the test procedure is valid and the sample well has a reading greater than or equal to 0.5.

STEP 11 : Confirming Positive Samples
Samples positive by the SURE *mono* ELISA kit should be confirmed by streaking them onto the selective plates.

STEP 12 : Disposal
When testing is completed, autoclave the wells used at 121°C for at least 30 minutes or incinerate as special waste.

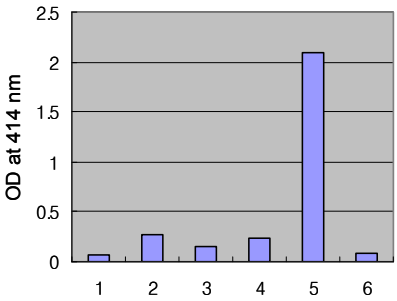
Specificity

Western blot
using the capture monoclonal antibody



M : marker
Lane1 : *L.grayi*
Lane2 : *L.welshimerii*
Lane3 : *L.innocua*
Lane4 : *L.ivanovii*
Lane5 : *L.monocytogenes*
Lane6 : *L.seeligerii*

SURE *mono* ELISA kit



1 : *L.grayi* (ATCC 25401)
2 : *L.welshimerii* (ATCC 35897)
3 : *L.innocua* (ATCC 33090)
4 : *L.ivanovii* (ATCC 19119)
5 : *L.monocytogenes* (ATCC 19115)
6 : *L.seeligerii* (ATCC 35967)

Procedure Summary

1. Prepare reagents and controls as instructed

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2. For samples and controls, conduct STEP 1 and 2

⇩

3. Add 100$\mu\ell$ positive control or negative control to each well
Add 200$\mu\ell$ sample to each well
Incubate at 37°C for 30 minutes
Aspirate and wash 3 times

⇩

4. Add 200$\mu\ell$ secondary antibody to each well
Incubate at 37°C for 30 minutes
Aspirate and wash 3 times

⇩

5. Add 200$\mu\ell$ Detector to each well
Incubate at 37°C for 30 minutes
Aspirate and wash 6 times

⇩

6. Add 100$\mu\ell$ Substrate solution to each well
Incubate at room temperature for 15 minutes

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7. Read at 414 nm

References

1. Beuchat, L. R., and J. H. Ryu. 1997. Produce handling and processing practices. Emerg. Infect. Dis. 3:459–465.

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3. Schlech, W. F. 1996. Pathogenesis and immunology of *Listeria monocytogenes*. Pathol. Biol. 44:775–782.

4. Schlech, W. F., III. 2000. Foodborne listeriosis. Clin. Infect. Dis. 31:770–775.

5. K-Y Yu, Y-S Noh, M-S Chung, H-J Park, N-S Lee, M-Y Youn, B-Y Jung and B-S Youn, 2004. Use of Monoclonal Antibodies That Recognize p60 for identification of *Listeria monocytogenes. CDLI* 11(3): 446-451.

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Troubleshooting Guide

.Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

AdipoGen Inc.

Room #401, Venture Building B,
Songdo TechnoPark, 7-50
Songdo-dong, Yeonsu-gu,
Incheon, Korea

TECHNICAL INFORMATION

Web www.adipogen.com

E-mail adipogen-kr@adipogen.com

Phone +82-32-858-1470

Fax +82-32-831-1470