For research use

Loopamp™

L. monocytogenes Detection Kit

[Characteristics]

LAMP (Loop-mediated Isothermal Amplification) method is a gene amplification method capturing the following characteristics: (1) Only one enzyme is required and the amplification reaction proceeds under isothermal condition[1)2)], (2) 4 primers recognizing 6 distinct regions on the target achieve high specificity, (3) High amplification efficiency allows amplification within a shorter time, (4) It produces tremendous amount of amplified products which makes simple detection possible[3)4)5)].

Amplification of nucleic acids with this kit is conducted by the LAMP method using primers which recognize the sequences *Listeria monocytogenes* (hereinafter referred to as *L .monocytogenes*) invasion gene (iap)[6),7)] . From whether the amplification occurred or not, the existence of *L. monocytogenes* can be determined.

By using the specifically designed Loopamp Realtime Turbidimeters, detection does not need electrophoresis and all steps from amplification to detection are done within one reaction tube. Simple and rapid detection of *L. monocytogenes* can be achieved[8),9)].

[Contents of the kit]

		48 tests
(1) Extraction Solution for Foods	(EX F)*1	1.8mL × 3 tubes
(2) 1M Tris-HCl: pH7.0	(Tris)*1	1.0mL × 1 tube
(3) Reaction Mix.Lis	(RM Lis)*1	1.0mL × 1 tube
(4) *Bst* DNA Polymerase	(Bst DNA Polymerase)*1	60μL × 1 tube
(5) Positive Control Lis	(PC Lis)*1	0.1mL × 1 tube

*1: The notation on each reagent tube is shown in ( )

[Intended use]

Detection of *L. monocytogenes* in foods or environmental specimens.

[Assay Principle]

This kit uses LAMP method as assay principle.

First, conduct enrichment culture of the food specimen and then after Alkalis heat extraction, the solution can be used as sample. Mix the sample solution with Reaction Mix. Lis (RM Lis) and *Bst* DNA polymerase, and incubate it. When *L. monocytogenes* invasion gene (iap) sequence that can be recognized by the primers exists, its DNA will be amplified with the activity of *Bst* DNA polymerase. The detection of nucleic acid is done by detecting the turbidity change caused by the amplification by-product magnesium pyrophosphate (white precipitate) and then determines whether there is *L. monocytogenes*.

For information on LAMP reaction principle, refer to Eiken GENOME SITE (URL: http://loopamp.eiken.co.jp/e/).

[How to use]

1. Materials required but not provided

- ○ Enrichment media (for pre-enrichment)
- ○ Stomacher bag with filter
- ○ Sterilized tubes for master mix preparation (0.5mL or 1.5mL)
- ○ Micropipette (0.5～10μL, 10～100μL, 100～1,000μL)
- ○ Pipette tips with filter
- ○ Sterilized tubes for pre-treatment of specimen (0.5mL)
- ○ Heat block (Use at 95°C)
- ○ Loopamp Reaction Tube
- ○ Aluminum rack for cooling tubes
- ○ Crushed ice and ice box
- ○ Loopamp Realtime Turbidimeter
- ○ Centrifuge for microtubes
- ○ Centrifuge for 8-strip tube
- ○ High-speed centrifuge (centrifuge for micro-tubes with 2,000×g or above can also be used)
- ○ Vortex mixer

2. Sample solution preparation

In the case of using pre-enrichment culture as specimen for the detection of *L .monocytogenes* in foods:

Food 25g + 225mL of Half Fraser media*2, UVM media*2 or EB media*2

→ Stomacher treatment →(Incubate at 30°C for 24 hours) → Pre-enrichment culture

*2: For different foods specimen, choose the suitable enrichment media accordingly.

1) Specimen pre-treatment (Preparing sample solution)

(1) Prepare necessary quantity of sterilized tubes for specimen pre-treatment, and dispense 50μL of pre-enrichment culture into each tube.

(2) Close the caps, centrifuge at 2,000×g for 5 minutes. Remove the supernatant carefully as to not convolute the precipitate. If there is no precipitate in the solution, remove 40μL of supernatant.

(3) Add 80μL of Extraction solution for Foods (EX F).

(4) Close the caps and mix by vortex mixer. Centrifuge for a few seconds (hereinafter referred to as spin down) and incubate at 95°C for 5 minutes.

(5) After incubation, spin down the tube and add 10μL of 1M Tris-HCl: pH7.0 (Tris).

(5) Close the caps and mix by vortex mixer. After centrifuge at 2,000×g for 30 seconds at room temperature, keep it on ice and use the supernatant as sample solution (remain stable at 0~4°C for 4 hours).

3. Preparing the reagents

1) Take out the reagents stored at -20°C, and thaw them at room temperature.
Once the reagents are thawed, keep them on ice.

2) Preparation of master mix (Operate on ice)

(1) Dispense the appropriate amount into the separately prepared sterilized tube under the proportion of Reaction Mix. Lis (RM Lis) 20μL and *Bst* DNA Polymerase 1μL per test (including positive and negative control tests).

(2) After dispensing, mix the solution by gently tap the tubes a few times (hereinafter referred to as tapping), or invert the tube, or by vortex mixer at about 1 second×3 times. After mixing well, spin down the tubes and the mixture can be used as the master mix for the reaction. Notice that too much mixing by the vortex mixer might inactivate the polymerase, and assure that vortexing is conducted at 1 second×3 times.

The prepared master mix should be used as soon as possible.

4. Operation procedure

1) Mixing Master Mix and sample solution (Operate on ice)

(1) Dispense 20μL of the master mix into each Loopamp Reaction Tube.

(2) Add 5μL of sample solution to the master mix and the volume of the solution should be 25μL in total. Mix the solution well by pipetting or tapping the tube with the cap closed and then spin down. Be careful not to cause air bubbles when mixing.

(3) For control reactions, use 5μL of Positive Control Lis (PC Lis) for positive control, and 5μL of Extraction Solution for Foods (EX F) for negative control.

2) Amplification reaction and realtime turbidity detection

(1) Loopamp Realtime Turbidimeters are applicable to this kit. Set the parameters as follows:
[Temperature]: Reaction Block 65ºC, Hot Bonnet 75ºC
[Measurement Time]: 60 min
[Inactivation]: 80ºC, 2 min

(2) Confirm that the temperature has reached 65ºC.

(3) Set the prepared reaction tubes and start reaction.

(4) Check whether the positive and negative controls turbidity rose from the display screen of the device. If the turbidity of positive control rose and the turbidity of negative control didn't, then LAMP reaction proceeded properly. If not, there might have been error in the process. Restart from reagent preparation and check again (Fig.1)

(5) Next, the judgment of each sample is conducted. If the increase in turbidity is confirmed within 60 minutes, then it is judged as positive, if not, negative. (Fig.2)

(6) The rising timing or turbidity of samples might be different from the positive control; Positive Control Lis (PC Lis).

(7) After enzyme inactivation (80ºC, 2min), done automatically by Loopamp Realtime Turbidimeters), is confirmed to have ended, remove the used reaction tubes and dispose them with caps closed.

■Amplification curve pattern

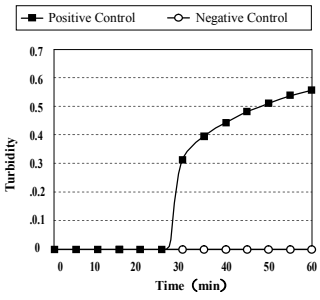

Fig.1 The amplification curve pattern of controls

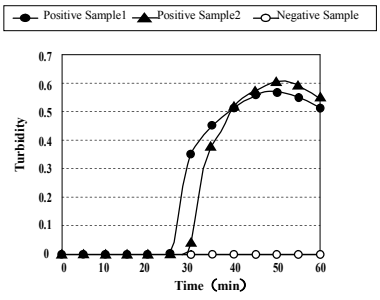

Fig.2 Samples amplification curve pattern*3

*3: This kit is not developed for the purpose of quantitative analysis, therefore, the copy numbers does not necessarily correlate with turbidity increment time.

[Caution for operation]

1. Sample handling

1) When conducting pre-enrichment, the stomacher bag with filter should be used.

2) When collecting pre-enrichment culture, pipette carefully as to not convolute the precipitate.

3) Basically, the sample (DNA extraction solution) should be used immediately. However, if it is to be stored for long period of time, it should be stored under -80ºC and repeated freezing and thawing should be avoided.

2. Reagent handling

1) This reagent kit should be stored at -20°C. To prevent the reagents from deterioration, only take out the necessary amount of reagents from the freezer before use. No decline was observed in the kit performance even after repeated freezing and thawing for 20 times in the quality control test. But, in order to maintain the reagents performance, avoid unnecessary freezing and thawing.

2) Thaw the reagents at room temperature and keep them on ice for reagents preparation. Before use, spin down the tubes to drop down the reagents staying on the tube wall or on the cap, mix well the reagents and spin down again. Notice that fierce mixing should be avoided as it can inactivate the *Bst* DNA polymerase.

3) Extraction Solution for Foods (EX F) gradually deteriorates when exposed to air. Opening and closing the cap of Extraction Solution for Foods should be limited as minimum as possible so that the time for exposing the solution to air can be limited as minimum as

possible. Please add Extraction Solution for Foods (EX F) as soon as possible for pre-treatment. When storing Extraction Solution for Foods, keep its cap tightly closed, and do not aliquot the solution.

4) Positive Control Lis (PC Lis) contains high number of copies. In order to prevent Positive Control Lis from contaminating other samples or reagents, always spin down before opening the tube and open the cap of the tube as shortly as possible. Also add into the reaction tubes under the following order from negative control (Extraction Solution for Foods (EX F)), sample solution (extracted DNA), and leave the adding of positive control (Positive Control Lis (PC Lis)) to the last and make sure that all other tube caps are closed when adding it. Moreover, to avoid contamination, do not use Positive Control Lis (PC Lis) in any other way not written in this instruction (such as diluting the positive control or adding it to samples).

5) Keep positive control and suspected positive samples away from the reagents when handling.

6) If there is any reagent left, do not use it with other kits even if they are in the same lot.

■ Protocol

<Pretreatment: DNA extraction solution preparation> (Operate on ice)

Dispense 50µL of Pre-enrichment culture or enrichment culture into the separately prepared pre-treatment tube.

⇩

Centrifuge at 2,000×G for 5 minutes. Remove the supernatant with pipette.

⇩

Add 80µL of Extraction solution for Foods (EX F).

⇩

Mix by vortex mixer. After spin down, incubate at 95°C for 5 minutes.

⇩

Add 10µL of 1M Tris-HCl:pH7.0 (Tris)

⇩

Mix by vortex mixer and centrifuge at 2,000×G for 30 seconds at room temperature.

⇩

Use the supernatant as sample solution (on ice)

<Master mix. preparation> (Operate on ice)
Prepare the necessary volume of master mix.[*5]

Master mix preparation (for 1 test)

Reaction Mix. Lis (RM Lis)	20µL
Bst DNA polymerase	1µL
Total	21µL

Mix thoroughly[*6], then spin down and use as master mix (on ice).

Procedure (Operate on ice)

Dispense 20µL of master mix. into each reaction tube.

Add 5µL of sample solution or control into tube.
Total of 25µL as LAMP reaction solution.

Add 5µL of Positive Control Lis (PC Lis) as Positive Control and 5µL of Extraction Solution for Foods (Ex F) as Negative Control

Mix the solution well by pipetting or tapping the tube with the cap closedand then spin down.

(Be careful not to course air bubbles when mixing)

<LAMP reaction>

Set the reaction tubes into the reaction blocks of Loopamp Realtime turbidimeter and start the reaction.

⇩ 65°C, 60 minutes

Enzyme inctivation (80°C, 2 minutes)

⇩ (Done by Loopamp Realtime Turbidimeters automatically)

Detection and judgment

[*4]: Sample solution can remain stable at 0~4°C for 4 hours
[*5]: It is recommended to prepare one test amount extra to the amount that is needed for the number of tests (including positive control and negative control).
[*6]: Mix by tapping, invert the tube or by vortex mixer at 1second×3 times.

3. Handling the reaction tube

1) Only use the specified Loopamp Reaction Tube for turbidity detection. Other reaction tubes might have different optical transparency and can cause misjudgment.
2) Take full care when handling reaction tubes, as they are vulnerable to scratches or damages.
3) Check carefully to see if the reaction tubes have any crack or scratch before use. Crack or scratch on the tube might not only cause false judgment but also contaminate the equipment. If the tubes are broken inside the reaction block of the Loopamp Realtime Turbidimeter, the reaction mixture can spill inside the equipment and cause unrecoverable contamination and malfunction.
4) By comparing the solution volume in all tubes, check visually if proper amount of sample solution and master mix has been dispensed into the reaction tube.

4. Caution for amplification reaction

Since bubbles in the solution will interfere the turbidity measurement and cause false judgment, try not to cause any bubble when mixing the master mix and the sample solution. If bubbles appear, spin down to remove them.

5. Caution for detection and judgment

1) Use only Loopamp Realtime Turbidimeter and Loopamp Reaction Tube for the assay.
2) Start up Loopamp Realtime Turbidimeter at least about 20 minutes before using it.
3) For judgment, check whether the turbidity of Positive Control Lis (PC Lis) has risen to determine whether the reagent is performing properly (if the nucleic acid amplification reaction is performed properly, the turbidity will start to rise around 30 minutes after the reaction started). There are cases where the turbidity of the sample starts rising later than the positive control.

6. Handling reaction tubes after use

1) The caps of the used reaction tubes should not be opened. Pay special attention not to accidentally open the cap when taking the tubes out of the turbidimeter. Contamination of amplified products on other samples may not only cause false judgment of the test result but also pollute testing area. In this case, a correct test result may not be obtained until the contamination is completely removed.
2) Keep the cap of the used tube completely closed and dispose it according to the relevant regulations and instructions by incineration or after double bagging it with sealable vinyl bag. To prevent the amplified products from dispersing, do not conduct autoclave sterilization treatment for disposal.

[Performance]

Sensitivity: 60 CFU (Colony Forming Unit)/ test[*7]

*7: As for *Listeria monocytogenes* ATCC19114, 600CFU/test

[Caution for handling]

1. LAMP reaction is very sensitive and even the slightest amount of amplified product tainted into the reaction might cause false result. Therefore, avoid this type of contamination by carrying out the sample and reagent preparation in different clean benches. Avoid electrophoresis or operations that need to handle amplified products.
2. About handling pre-enrichment media, refer to their instruction manuals. When handling the sample, always abide by the biohazard counter measures [10].
3. Do not expose the Loopamp Reaction Tube, master mix preparation tubes to UV light. A change in color or deterioration caused by ultraviolet lamp sometimes results in misjudgment.
4. This kit is for the purpose of foods and environment inspection, not for medical or clinical diagnostic purposes on human or animal samples.
5. If the operator does not have the experience or knowledge in the field of nucleic acid testing, there is a possibility of false judgment. Therefore, make sure that the kit is used under the supervision of the experienced and knowledgeable technicians.
6. This kit can detect *L.monocytogenes* through amplifying its gene, which is different from the conventional culture method that can only detect viable bacteria of *L.monocytogenes*. Use this kit as a part of self-imposed test.
7. The result of this kit might be different from that of the culture method.
8. Eiken Chemical Co., Ltd. does not bear any responsibility for false judgment or any consequential damage derived from the false judgment caused by non-capability problems such as operation error.
9. Use the kit before the expiration date, which is labeled on the outer box (Exp. Date).
10. This kit should be stored at -20°C. Storing the kit at temperature lower than -20°C or repeated freezing and thawing might cause cracks on the tube of 1M Tris-HCl: pH7.0 (Tris).
11. The reagent tube is made of polypropylene and the main material for kit case is paper. The institution disposing the reagent tube and case should bear the responsibility and abide by the clinical waste disposal regulations, water pollution prevention law, and any other regulations related.

[Unit, Storage, Expiration, Code No.]

Product Name	Unit	Storage	Expiration	Code No.
Loopamp™ *L.monocytogene* Detection Kit	48 tests	-20 °C	1 year	LMP701

[References]

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