Assurance® GDS *Listeria monocytogenes* Tq

MicroVal Certificate No. 2014LR32

Part No: 71010-100 (100 tests)
71010-576 (576 tests)
71010-576ATM (576 tests)

General Description

Assurance® GDS, genetic detection system, for *Listeria monocytogenes* Tq is an automated nucleic acid amplification system for the detection of *Listeria monocytogenes* from food and environmental samples.

Kit Components

Each Assurance® GDS *Listeria monocytogenes* Tq kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent
- *Listeria* Resuspension Buffer Tq
- Wash Solution

Each Assurance® GDS for *Listeria monocytogenes* Tq 576ATM kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary but sold separately:

61031-100 Wash Solution Kit
34745-100C *Listeria* Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- Enrichment Media (see Appendix A)
- Assurance® GDS Rotor-Gene®
- PickPen® and PickPen® tips
- Vortex mixer
- Adhesive film
- Sample wells and sample wells base
- Resuspension plate
- Gel cooling block
- Stomacher / Masticator or equivalent
- 8-channel micropipette capable of dispensing 30 µL
- Repeat pipette
- Adjustable micropipettes

Repeat pipette tips (0.5 mL and 10 mL)

Filter barrier micropipette tips (50 µL and 1.0 mL)

Incubators capable of maintaining and 30 °C and 60 °C

Additional materials for the 576 kit include:

Variable Spacing Multi-Channel Pipette

Aluminum Cooling Block, 72 well

72-well rotor and locking ring

MicroVal Method 2014LR32

Approved categories include: Meat Products, Milk & Dairy Products, Fish & Seafood Products, RTE or to Reheat Products and Environmental Samples

Sample Preparation

A. Test Portion Preparation & Enrichment

a. For **foods**, add 25 g of sample to 225 mL of pre-warmed (30 °C) Demi or Half Fraser Broth (Appendix A). Homogenize if necessary.

b. For **environmental monitoring**, collect environmental surface samples with a sponge or swab hydrated with D/E (Dey/Engley) Broth or Letheen Broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of pre-warmed (30 °C) Demi Fraser Broth. (Appendix A). Masticate to mix well.

Note: Sponges and swabs hydrated with Neutralizing Buffer are not recommended for use with Assurance® GDS for *Listeria monocytogenes.*

c. Incubate samples for 22 – 26 h at 30 °C.

B. Sample Preparation

Change gloves prior to handling Reagents

a. Vortex **Concentration Reagent.** Immediately dispense 20 µL to each of the required number of Assurance® GDS sample wells (1 well/sample) using a repeat pipettor and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.

b. Dispense 1.0 mL of **Wash Solution** to 2 additional sets of sample wells (2 wells/sample) using a repeat pipetter and 10 mL pipette tips. Cover sample wells with adhesive film strips.

c. Transfer 0.5 mL of fresh, sterile Demi or Half Fraser Broth to additional sample wells (1 well/sample) using a repeat pipettor and 10 mL pipette tips.

d. Dispense 45 µL of **Resuspension Buffer Tq** to the wells of the resuspension plate using a repeat pipettor and a 0.5 mL pipette tip. Cover prepared resuspension plate with adhesive film strips.

e. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip.

f. Place sealed sample wells containing Concentration Reagent and sample on the vortex mixer and vortex at 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

g. Carefully remove and discard adhesive film strip from a strip of samples.

h. Load tips onto the PickPen®, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

i. Remove adhesive film strip from one set corresponding wells containing Wash Solution. Transfer PickPen® to the Wash Solution. With tips submerged, gently stir the PickPen® from side to side for 10 s. Tap the PickPen® tips against the side of the sample wells to remove excess wash solution droplets.

j. Remove adhesive film strip from the corresponding wells containing fresh Demi Fraser Broth. Transfer PickPen® to the Demi Fraser Broth. With tips submerged, retract PickPen® magnets and mix gently to release particles into media.

k. Cover wells and incubate particles and media for 4 – 22 h at 30 °C.

Note: All meat samples must be incubated a full 20 – 24 h.

l. Following incubation, carefully remove and discard adhesive film strip from a strip of samples.

m. Load tips onto the PickPen®. Extend the PickPen® magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

n. Remove adhesive film strip from the second set of corresponding wells containing Wash Solution. Transfer PickPen® to the Wash Solution. With tips submerged, gently stir the PickPen® from side to side for 10 s. Tap the PickPen® tips against the side of the sample wells to remove excess wash solution droplets

o. Remove adhesive film strip from resuspension plate. Transfer PickPen® to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the Resuspension Buffer Tq.

p. Repeat steps (l) through (o) for all samples using new tips for each strip of samples.

q. Cover resuspension plate with adhesive film strips.

r. Place sealed resuspension plate containing samples in 60 °C incubator for 15 min – 1 h.

Test Procedure

A. Preparation of Gel Cooling Block

a. Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15 °C) for 6 h. When frozen the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -25 to -15 °C.

b. Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

c. The aluminum cooling block is for use with the 576 test kit and should be stored in the refrigerator (2 – 8 °C). To use, place the refrigerated aluminum cooling block on top of the frozen gel cooling block.

B. Preparation of Amplification Tubes

a. The Assurance® GDS Rotor-Gene set up and data entry should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes.

b. Remove **Amplification Tubes Tq** from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for 576 test kit). Reseal pouch.

c. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipettor and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.

d. Place Amplification Tubes into Assurance® Rotor-Gene in sequential order, beginning with position #1. For the 100 test kit, use the 36-well rotor and locking ring; for the 576 test kit, use the 72-well rotor and locking ring.

Note: For 576 test kit, after loading amplification tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note FRMMK.2060 for details.

e. Start Rotor-Gene cycle. Refer to Assurance® GDS user manual for detailed instructions on operating the Rotor-Gene.

Note: The Assurance® GDS Rotor-Gene must be started within 20 min after addition of the samples to the Amplification Tubes.

Results

Upon completion of the run, the Assurance® GDS Rotor-Gene software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp.**

No.	Color	Name	Result	Assay	Kit Lot Number
1		Sample 1	Positive	*Listeria monocytogenes*	1234567
2		Sample 2	Negative	*Listeria monocytogenes*	1234567
3		Sample 3	No Amp	*Listeria monocytogenes*	1234567

Positive: Samples are positive for *L. monocytogenes*

Negative: Samples are negative for *L. monocytogenes*

No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact BioControl Technical Service.

Confirmation

Samples can be confirmed from the initial Demi Fraser Both enrichment following EN ISO 11290-1/A1 (2004): Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: detection of *Listeria monocytogenes* in foods.

Storage

Store Assurance® GDS for *Listeria monocytogenes* Tq kit components at 2 – 8 °C. Kit expiration is provided on the product box label.

Precautions

If possible, maintain separate work zones and dedicated equipment and supplies for sample preparation and amplification and detection.

It is recommended to utilize both positive and negative control samples.

This product is not intended for human or veterinary use. Assurance® GDS for *Listeria monocytogenes* Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

Do not use test kit beyond expiration date on the product box label. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state and federal regulations.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab.

If contamination is suspected, moisten paper towel with bleach solution and wipe all lab benches and equipment surfaces with 10% bleach solution. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl alcohol solution add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol. Waste may be contaminated with *Listeria* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.
Pregnant women, elderly, and potentially immune-compromised individuals must be prohibited from laboratory rooms or areas where *Listeria monocytogenes* enrichment, isolation, and identification procedures are in progress.

Although a properly sanitized laboratory area should not harbor *Listeria*, supervisors should use their own discretion in allowing these high-risk individuals into these areas.

APPENDIX A – Enrichment Protocols and Media

Demi or Half Fraser Broth Base

Suspend 55 g of dehydrated Demi-Fraser Broth Base in 1.0 L of purified water. Mix thoroughly, dispense into desired aliquots and autoclave at 121 °C for 15 min. Pre-warm to 30 °C prior to use.

Demi or Half Fraser Broth Base Ingredients

Formula per Liter	
Tryptose	10.0 g
Beef extract	5.0 g
Yeast extract	5.0 g
Sodium chloride	20.0 g
Disodium phosphate	9.6 g
Monopotassium phosphate	1.35 g
Esculin	1.0 g
Nalidixic Acid	0.01 g
Acriflavine HCl	12.5 mg
Lithium Chloride	3.0 g

Manufacturing Entity

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