



## **NON-CLINICAL RESEARCH USE ONLY**

InstantLabs®

*Listeria monocytogenes* Food Safety Kit

P/N 9034-0600-0010

### **PRODUCT DESCRIPTION**

The InstantLabs Medical Diagnostics Corporation (InstantLabs) *Listeria monocytogenes* Food Safety Kit contains the reagents necessary for performing qualitative, real-time PCR on the Hunter Accelerated PCR® system (see Hunter System Specifications below). The kit is intended to be used in an analytical laboratory under standard laboratory conditions.

### **KIT STORAGE AND STABILITY**

Store the InstantLabs® Food Safety Kit at 2 - 8° C. The reagents are stable through the expiration date printed on the kit.

### **MATERIALS REQUIRED AND PROVIDED**

- Multiple Assay Cartridges (MAC)
- Reagent Packs each containing the following:
  - 6 – Colored Tubes With Lyophilized PCR Reagents
  - 4 – Sample Extraction Tubes
  - 1 – Tube of Positive Control DNA
  - 1 – Tube of Nuclease-Free Water

Quantitative PCR complete master mix containing GoTaq® Hot Start Polymerase manufactured by Promega Corporation for distribution by BioGX, Inc. Licensed to Promega under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents. For Research Use Only. Not for any clinical or therapeutic use in humans or animals.

## **ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED:**

1. Hunter Accelerated PCR® Instrument
2. Nitrile or Latex Gloves
3. Whirl-Pak® Filter Bag for Homogenizer Blenders (or Other Similar Filter Bag)
4. Buffered *Listeria* Enrichment Broth (InstantLabs) or Other Suitable *Listeria* Selective Enrichment Media
5. 10-100 µL Pipette with RNase/DNase-Free Filter Tips
6. Heat Block Set at 95° C
7. Incubator Set at 30° C
8. Micro-Centrifuge Capable of at Least 6,000xg
9. Vortex w/1.5 mL Tube Adaptor

### **For Liquid Samples Only:**

10. Sterile Transfer Pipet of Suitable Capacity

### **For Solid Samples Only:**

11. Sterile Spatula (Single-Use or Laboratory-Sterilized)
12. Laboratory Balance with a Sensitivity of at Least 1g

## WARNINGS AND PRECAUTIONS

1. Wear eye protection, gloves, and laboratory coats when handling samples and kit reagents.
2. Avoid contamination of reagents by only using sterile pipette tips.
3. Do not use kits beyond the printed expiration date.
4. Do not use kits that have been stored above 8°C.
5. A negative and a positive control must be included in each test.
6. Do not reuse excess reagents from one reagent pack to another, dispose of all remaining reagents once all kit MACs have been used.
7. Material Safety Data Sheets (MSDS) are available upon request.
8. Treat enriched samples as if they contain pathogenic bacteria.
9. Enriched samples and MACs should be disposed of as bio-hazardous waste according to local regulations.

# INSTRUCTIONS FOR USE

## FOR TESTING OF FOOD SAMPLES (25 g)

### Sample Collection, Homogenization, and Enrichment

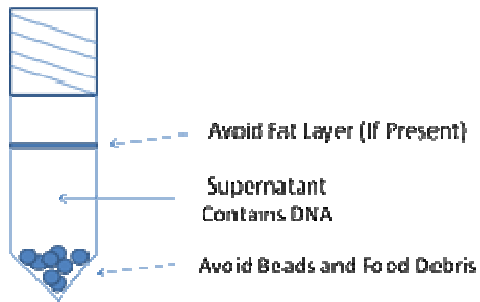
1. Prepare 225 mL of sterile Buffered *Listeria* Enrichment Broth (BLEB) for each sample to be tested by following the manufacturer's instructions. *Ensure that the temperature of the broth is at approximately room temperature immediately before use.*
2. Clean the work area and weighing scale with a 10% bleach solution and wipe until dry.
3. Using clean disposable gloves, carefully open and place a new Whirl-Pak bag onto the laboratory scale.
4. Aseptically transfer approximately 25 g of a representative food sample to the bag using a sterile spatula or scoop. For liquid samples, transfer 25 mL using a sterile pipet. *It is important to try to work quickly to minimize the chances of contaminating the sample.*
5. Repeat steps 3 and 4 (above) for each additional sample to be tested.
6. Pour 225 mL of BLEB with Supplements into each bag and loosely seal bag with built-in wire closures. *If necessary, sample size may deviate from 25 g depending on availability of the sample, as long as the amount of BLEB is adjusted proportionally.*
7. Gently hand massage Whirl-Pak bag(s) for 1 – 2 minutes to mix contents. *Samples may also be processed using a Stomacher machine for 30 – 90 seconds at maximum speed; this is recommended for all meat samples.*

8. Incubate the Whirl-Pak bag(s) without shaking at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for  $24 \pm 2$  hours

## DNA Extraction from Enriched Samples

1. Clean the work area and laboratory pipets with a 10% bleach solution and wipe until dry.
2. Firmly tap Sample Extraction tubes on the laboratory bench to collect contents at bottom of tube. Label each tube with identifying information for the sample to be tested.
3. Remove Whirl-Pak bag(s) from incubator being careful not to disturb the settled food debris.
4. Carefully open the bag and remove 50  $\mu$ L of liquid from the top 1 – 3 cm of the filtered compartment of the Whirl-Pak bag without disturbing the settled food debris and place into the correctly labeled Sample Extraction tube prepared in steps 2 above. *Note that if a layer of fat is present, move pipet tip to below the fat layer before removing aliquot.*
5. Repeat step 4 for each sample to be tested.
6. Gently invert Sample Extraction tube(s) 3 – 5 times to ensure sample is mixed with the lysis reagents.
7. Vortex Sample Extraction tubes at maximum speed for 10 minutes.
8. Heat Sample Extraction tubes to 95°C for 20 minutes in a dry heat block to complete the lysis. Let tube(s) cool on ice for 1 - 2 minutes.
9. Centrifuge tube(s) in a Micro-Centrifuge for 2 minutes at maximum speed to pellet the lysing beads and any food debris.
10. The **supernatant** contains the extracted DNA and is ready to be tested immediately with the InstantLabs® Food Safety Kit. *If the samples will not be tested within 30 minutes, transfer 50  $\mu$ L of supernatant to a new nuclease-*

*free microcentrifuge tube and freeze at -20° C for up to 3 months.*



Graphic of Sample Extraction Tube Following Centrifugation

## PCR Setup

### Overview:

Each Reagent Pack and Multiple-Assay Cartridge (MAC) can be used to run up to 4 samples plus the required positive and negative controls. The tubes in the reagent pack are color-coded to match the wells on the MAC and contain convenient ready-to-use lyophilized reagents:

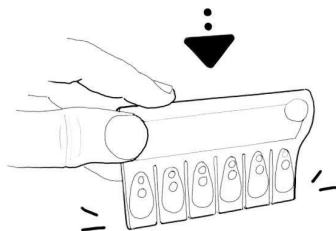


### Procedure:

1. Clean workspace and pipets with a 10% bleach solution and wipe until dry.
2. Using clean gloves remove one Reagent Pack from the kit.
3. Transfer 25  $\mu$ L of the extracted DNA sample to be tested to the green colored tube using a sterile pipet while being careful to not touch the lyophilized reagents with the pipet tip. Shake tube gently to ensure that lyophilized reagents are reconstituted.
4. Additional extracted samples (up to a total of 4) can be similarly loaded into the Orange, Purple, and Yellow tubes if desired. Shake the tubes gently to ensure that lyophilized reagents are reconstituted.
5. Using a sterile pipet transfer 25  $\mu$ L of Nuclease-Free Water to the blue colored Negative Control tube. Shake tube to ensure that lyophilized reagents are reconstituted.



6. Using a sterile pipet transfer 50 uL of Nuclease-Free Water to the tube labeled Positive Control and shake tube vigorously for 15 sec to mix contents. Using a new pipet tip transfer 25 uL of this solution to the red colored Positive Control tube. Shake tube gently to ensure that lyophilized reagents are reconstituted.
7. Briefly spin down the 6 colored tubes in a Mini-Centrifuge for 15 seconds to collect contents at bottom of tube.
8. Remove one MAC from the kit.
9. Prepare Hunter instrument by entering test and sample information as outlined in the Hunter User Manual.
10. Pipette 20µl of each Sample/Control PCR mix solution to the correct port on the MAC by matching the color of the tube to the colors printed on the MAC. Ensure that the solution flows into the sample chamber and then close the cap on the port. To ensure a proper seal press the cap down firmly.
11. When all samples have been added to the MAC, tap the MAC gently on the countertop to ensure there are no visible bubbles present in the sample chamber.



12. Place the MAC into Hunter and firmly push latch down to lock MAC in place.
13. Close the Hunter's door to start the test which will begin in 15 seconds.

14. The results will be displayed automatically as Positive, Negative, or Indeterminate when the test is completed.

## TROUBLESHOOTING

Problem	Suggestion
No amplification of Positive Control – The Hunter calls the positive control Negative or Indeterminate	<ul style="list-style-type: none"><li>• Positive Control was expired, not reconstituted properly, or was not loaded into the MAC correctly. Repeat test with fresh reagents.</li></ul>
High background in the Negative Control – Hunter calls all samples indeterminate (?)	<ul style="list-style-type: none"><li>• Potential contamination. Repeat test with fresh reagents.</li></ul>

# HUNTER SYSTEM SPECIFICATIONS

## System Components

- Hunter device
- MAC Assay Cartridges (MAC)

## Additional Components

- Hunter device User Manual CD
- 2 spare fuses
- AC line cord

## Hunter instrument specifications

- Dimensions: 17.2 cm x 41.2 cm x 22 cm (6.77 in. H x 16.22 in. W x 8.66 in. D)
- Weight: 5.4 Kg (11.9 lbs.)
- Power usage: 90 to 264 VAC at 3 amps
- Fuses (2): 3 AMP slow blow
- Peak block heat rate ~ 4.5°C/sec
- Peak block cool rate ~ 4.5°C/sec
- Warm up time: ~10 minutes
- Decibel level: less than 40 db at 1 meter

## Environment

- Temperature 10-35° C (50°-95°F)
- Relative humidity up to 90% (noncondensing)
- Locate away from heaters, cooling ducts and keep out of direct sunlight
- Protect from all fluids

## Throughput

- Six (6) samples every 2.25 hours (run time)
- Includes pos/neg control
- 18 samples per 8 hour shift
- Supported volumes 20ul

## Area Requirements

- 6" recommended free space around the Hunter system for sample preparation, airflow, etc.

## TECHNICAL ASSISTANCE

Contact Your Local Distributor for Technical Assistance

Worldwide Support:

<b>Phone:</b>	703-904-4306
<b>Email:</b>	<a href="mailto:support@instantlabs.com">support@instantlabs.com</a>
<b>Web:</b>	<a href="http://www.instantlabs.com">www.instantlabs.com</a>

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