Read instructions carefully before starting test

GeneQuence® *L. monocytogenes*

DNA Hybridization Test for Detection of *Listeria monocytogenes*

INTENDED USE

This test is intended to qualitatively detect *Listeria monocytogenes* (*L. mono*) in food and environmental samples, and should be used by personnel with appropriate laboratory training in microbiology.

TESTING DIFFERENT COMMODITIES

GeneQuence *L. mono* Test is an effective screening tool for a variety of commodities. Through evaluation by the AOAC Research Institute, this test has been shown to detect *Listeria monocytogenes* in the following food products: Deli turkey, deli ham, deli roast beef, hot dogs, raw ground beef, raw ground pork, smoked salmon, cooked crab meat, raw shrimp, ice cream, pasteurized milk, brie cheese, parmesan cheese, lettuce, frozen peas, and soy flour.

BIOLOGICAL PRINCIPLES OF THE TEST

This DNA hybridization test employs *L. mono*-specific DNA probes directly labeled with horseradish peroxidase, and a colorimetric endpoint to detect as little as one colony forming unit (CFU) of *L. mono* in a 25 g food sample when the specified enrichment protocols are used. A sample is considered negative for the presence of *L. mono* if the absorbance value (A_{450}) obtained is less than 0.10. A sample is considered positive for the presence of *L. mono* if the absorbance value obtained is greater than or equal to 0.10. Positive samples should be confirmed by standard culture procedures, followed by biochemical identification.

MATERIALS PROVIDED

1. 1 bottle of pretreatment concentrate (labeled 1a)
2. 1 bottle of 12 mL pretreatment buffer (labeled 1b)
3. 1 bottle of lysis reagent concentrate (labeled 2a)
4. 1 bottle of 12 mL lysis reagent buffer (labeled 2b)
5. 1 bottle of 18 mL hybridization solution (labeled 3)
6. 1 bottle of 5 mL *L. mono* probe solution (labeled 4)
7. 1 bottle of 50 mL wash solution 20X concentrate (labeled 5)
8. 1 bottle of 15 mL substrate chromogen solution (labeled 6)
9. 1 bottle of 5 mL stop solution (labeled 7)
10. 1 bottle of 5 mL positive control (labeled +)
11. 1 bottle of 5 mL negative control (labeled -)
12. 1 96-well microwell plate in divisible strips
13. Hybridization/probe mixture dilution chart

MATERIALS REQUIRED BUT NOT PROVIDED

AVAILABLE FROM NEOGEN

EQUIPMENT

1. Angled thermometer for digital heat block (item #9382)
2. Apparatus for plate washing with vacuum source (Manual item #9348, Automated item #6718 or #6718A)
3. Automated plate wash assembly: vacuum tubing assembly (item #9387), side arm flask assembly (item #6822), vacuum pump (item #6824)
4. Carboy w/spigot (2) (item #9349)
5. Digital heat block (item #9386)
6. Heated block cover (item #9383)
7. Incubators at 30°C ± 1° and 35°C ± 1° (item #9735)
8. Microwell reader to read at 450 nm with discrimination of 0.01 absorbance unit (item #6704, #6704A or #9302)
9. Modular heating block titer plate (item #9384)
10. Water bath at 37°C (item # 9346)

OPTIONAL EQUIPMENT

1. Dry heater base incubator (item #9412)
2. 8-channel pipettor & tips to dispense 50-300 µL (item #9385, #9407)
3. Heater block at 37°C ± 1° (item #9411)

LAB SUPPLIES & CONSUMABLES

1. Micropipette & tips to dispense 20-200 µL volumes (item #9276, #9407)
2. Micropipette & tips to dispense 200-1000 µL volumes (item #9342, #9343)
3. Minute timer (item #9426)
4. Test tubes, 12 x 75 mm borosilicate glass, and rack (items #9439 and #9440)
5. Stomacher bags(100) (item #9736)
6. 50 mL culture tubes for sample pre-enrichment (item #9381)
7. 1000 mL graduated cylinder (item #9345)
8. 500 mL wash bottle (item #9366)
9. 10 mL pipette pump (item #9277)

10. 10 mL sterile serological pipettes, 0.1 mL graduations (item #9415)
11. Eppendor reservoir (item #4018)
12. Centrifuge tube racks (item #9479)

MEDIA

1. BLEB Base Supplements (item #7980)
2. Buffered Listeria Enrichment Broth Base (BLEB Base, item #7675)
3. LESS Enrichment Broth (item #9790A)
4. Oxford Listeria Agar Base (item #7428)
5. Oxford Medium Antimicrobics, FDA (ref. 1, item #7986)
6. PALCAM Broth Base (ref. 3, item #7670)
7. PALCAM Supplement (ref. 3, item #7987)
8. UVM Modified Listeria Enrichment Broth, UVM (ref. 2, item #7409)

STARTER KITS

1. GeneQuence Starter Kit, includes all items (item #9268)
2. GeneQuence Starter Kit, without water bath (item #9268a)
3. GeneQuence Consumables Starter Kit (item #9266)
4. GeneQuence Small Wash Starter Kit (item #9267)

NOT AVAILABLE FROM NEOGEN

1. Homogenizer (e.g., Stomacher)
2. Modified Oxford Medium (MOX), FSIS (ref. 2)
3. Petri plates, 100 x 15 mm
4. Phosphate-Buffered Saline (PBS), 10 mM sodium phosphate, 0.85% sodium chloride, pH 7.4
5. Sodium Pyruvate Supplement (10% aqueous solution, ref. 1)
6. Sterile cotton swabs and absorbent paper
7. 2 mL disposable graduated pipettes

PRECAUTIONS

1. Reagents are for laboratory use only.
2. Stop solution contains 4.0 N sulfuric acid. Hybridization solution contains formamide. Avoid contact with skin and mucous membranes. Refer to the Material Safety Data Sheet available from Neogen for more information.
3. Reagents should not be used beyond their expiration dates.
4. Reagents from different kit lots should not be interchanged. GeneQuence *L. mono* Test reagents are not interchangeable with other GeneQuence assay reagents.
5. Enriched cultures should be handled/disposed of as potentially infectious material.
6. The preferred method for disposal of contaminated materials, including cultures, pipettes, etc., is autoclaving.
7. Items that cannot be autoclaved should be decontaminated by treatment with a disinfectant solution and rinsing with water.
8. This test should be performed in a normal laboratory environment with respect to humidity, lighting, etc. Steps requiring room temperature incubation should be performed at 18-30°C.
9. Pregnant women and immunocompromised individuals should exercise extreme caution when working with materials that may contain *Listeria* spp. Consult with the safety director of your facility for specific instructions.

SAMPLE PREPARATION AND ENRICHMENT

Notes: *Food samples should be obtained and handled according to standard practices appropriate to analysis for* L. mono *(refs. 1-2). All enrichment media should be prewarmed to room temperature before inoculation. If needed, media formulations are available from Neogen.*

Raw and cooked meats and poultry:

1. Homogenize (Stomacher, 1-2 min.) 25 g sample in 225 mL UVM Broth. Incubate 24 ± 2 hours at 30°C.
2. Remove UVM culture from incubation and mix well. Transfer 0.1 mL UVM culture to 10 mL PALCAM Broth. Incubate 24 ± 2 hours at 35°C.
3. Perform assay on 0.4 mL aliquot of PALCAM culture. Save PALCAM culture for possible confirmation.

Cooked seafoods, dairy products, and fruits and vegetables:

1. Homogenize (Stomacher, 1-2 min.) 25 g sample in 225 mL Buffered *Listeria* Enrichment Broth Base (BLEB Base) supplemented with 0.1% (w/v) Sodium Pyruvate. Incubate 4 hours at 30°C. Add BLEB supplements and incubate 20 ± 1 hour at 30°C.
2. Remove BLEB culture from incubation and mix well. Transfer 1 mL BLEB culture to 9 mL BLEB with supplements. Incubate 24 ± 2 hours at 35°C.
3. Perform assay on 0.4 mL aliquot of secondary BLEB culture. Save secondary BLEB culture for possible confirmation.

Raw seafoods:

1. Homogenize (Stomacher, 1-2 min.) 25 g sample in 225 mL Buffered *Listeria* Enrichment Broth Base (BLEB Base) supplemented with 0.1% (w/v) sodium pyruvate. Incubate 4 hours at 30°C. Add BLEB supplements and incubate 20 ± 1 hour at 30°C.
2. Dip a sterile cotton swab into culture and swab onto the entire surface of an OXA plate, expressing as much liquid from the swab as possible. Incubate plate 24 ± 2 hours at 35°C.
3. With a sterile cotton swab, swab off growth from the plate (swab entire surface of plate, removing as much growth as possible) and suspend in 1 mL Phosphate Buffered Saline in a sterile, capped tube by swirling swab vigorously for 5 seconds. Express as much liquid as possible before discarding the swab.
4. Perform assay on 0.4 mL aliquot of growth suspension. Save growth suspension for possible confirmation.

Environmental swab samples:

1. Place swab in 10 mL UVM Broth. Vortex or mix vigorously for 10 sec. Incubate 24 ± 2 hours at 30°C.
2. Remove UVM culture from incubation and mix well. Transfer 0.1 mL UVM culture to 10 mL PALCAM Broth. Incubate 24 ± 2 hours at 35°C.
3. Perform assay on 0.4 mL aliquot of PALCAM culture. Save PALCAM culture for possible confirmation.

Environmental sponge samples:

1. Place sponge in appropriate volume (e.g., 200 mL) UVM Broth. Vortex or mix vigorously for 10 sec. Incubate 24 ± 2 hours at 30°C.
2. Remove UVM culture from incubation and mix well. Transfer 0.1 mL UVM culture to 10 mL PALCAM Broth. Incubate 24 ± 2 hours at 35°C.

3. Perform assay on 0.4 mL aliquot of PALCAM culture. Save PALCAM culture for possible confirmation.

SINGLE-STEP ENRICHMENT

All foods:

1. Homogenize (Stomacher, 1-2 minutes) 25 g sample in 225 mL LESS Enrichment Broth. Incubate 27-30 hours at 30°C (minimum 30 hours for ice cream, crab meat, and lettuce).
2. Perform assay on 0.4 mL aliquot of LESS Broth culture. Save culture for possible confirmation.

Environmental swab samples:

1. Place swab in 10 mL LESS Enrichment Broth. Vortex or mix vigorously for 10 seconds. Leave swab in broth. Incubate 24-48 hours at 30°C.
2. Perform assay on 0.4 mL aliquot of LESS Broth culture. Save culture for possible confirmation.

Environmental sponge samples:

1. Place sponge in appropriate volume (e.g., 100-200 mL) LESS Enrichment Broth. Stomach or mix vigorously for 10 seconds. Leave sponge in broth. Incubate 24-48 hours at 30°C.
2. Perform assay on 0.4 mL aliquot of LESS Broth culture. Save culture for possible confirmation.

PRIOR TO STARTING THE TEST

1. Allow the refrigerated reagents to equilibrate to room temperature.
2. Turn on the water bath or heater block and adjust to 37°C ± 1°. Fill a water bath to a level of approximately 1.5 inches, or fill the heater block wells about 1/3 with deionized water. Turn on the microwell plate heater block and adjust to 45°C ± 1°.
3. Prepare pretreatment reagent by adding 12 mL of pretreatment buffer (bottle 1b) directly to the pretreatment concentrate (bottle 1a). Dissolve contents by gently swirling.
4. Prepare lysis reagent by adding 12 mL of lysis reagent buffer (bottle 2b) directly to the bottle of lysis reagent concentrate (bottle 2a). Dissolve contents by gently swirling.
   Note: *The pretreatment reagent prepared in step 3 and lysis reagent prepared in step 4 may be combined in bottle 2a. Separate pretreatment and lysis reagents, or the reagents combined in bottle 2a, are stable in the reconstituted form for 60 days when stored at -20°C. To thaw, place bottles at room temperature. When thawed, gently swirl contents. Return reconstituted reagents to storage at -20°C immediately after each use.*
5. For each sample to be tested, label a 12 x 75 mm glass test tube with the appropriate sample designation and place in a rack. Include tubes for 1 positive control and 1 negative control per experimental run.
6. Prepare the wash solution by mixing entire contents of wash solution concentrate (bottle 5) with 950 mL of distilled or deionized water (if washing manually with a 500 mL wash bottle, use 25 mL of concentrate with 475 mL of water). Fill the buffer reservoir of the plate-washing device (see manufacturer's instructions for set-up and use). **Note:** *Wash solution can be stored in a closed bottle at room temperature for up to 60 days.*

7. Without touching the bottoms of the wells, place the appropriate number of microwells in the plate frame, filling the frame front to back, left to right, in rows of 8. Include wells for the reagent blank, negative control and positive control. **Note:** *If the last row has fewer than 8 wells and a plate-washing device is used, fill the last row with colored wells (colored wells are available free from Neogen).*

TEST PROCEDURE

Note: *This test is adaptable to automation. Contact Neogen for further information.*

1. Mix the test samples by gently shaking or vortexing the culture tubes. **Shake the positive and negative control solutions by inverting the bottles several times.** Add 0.4 mL of each control and test sample to the appropriately labeled tubes.
2. Add 0.1 mL of reconstituted pretreatment reagent (bottle 1a) to each tube and 0.1 mL of reconstituted lysis reagent (bottle 2a). Optionally, add 0.2 mL of the combined pretreatment/lysis solution (bottle 2a) to each tube. Mix by gently shaking the rack of tubes by hand for 5 seconds. The resulting solution should be green (level depends upon food commodity). If any tubes are not green, check for proper reagent addition. Incubate the rack of tubes in the 37°C water bath or heater block for 5 minutes.
3. Prepare a 3:1 hybridization/probe mixture by mixing hybridization solution (bottle 3) and probe solution (bottle 4) in a plastic container. For mixture guidelines, refer to the mixing chart provided with this test kit or use the formula below. (N = number of test samples + controls)

   Volume hybridization solution (bottle 3) = [(N x 0.09) + 1.5] mL
   Volume probe solution (bottle 4) = [(N x 0.03) + 0.5] mL
4. Remove the tubes from the heat source. With the microtiter plate on the 45°C heater block, transfer 0.150 mL of each lysed sample, including the controls, to designated microwells. The first well should be reserved for the reagent blank and receives no sample. The second well should be used for the negative control, and the third for the positive control.
5. Vigorously mix the hybridization/probe solution prepared earlier. With the microtiter plate still on the 45°C heater block, add 0.125 mL to each microwell, and mix each well 10 times with the pipettor. Do not add hybridization/probe solution to the reagent blank microwell.
6. Cover the heater block and incubate the plate at 45°C for 60 minutes.
7. Wash the wells using one of the following procedures:
   a) **Plate-washing device:** Wash 5 times at room temperature. For each wash, process one 8-well strip at a time by aspirating the liquid, filling the wells, and then proceeding to the next strip. After the last wash, aspirate the liquid from the wells, then remove any residual liquid by inverting the plate and tapping it onto absorbent paper. Hold the plate by gently squeezing on the sides of the frame to keep the strips in place. Be sure to not touch the bottom or sides of wells with plate washer.
   b) **Manual:** Thoroughly wash the microwells at least 5 times using a plastic wash bottle filled with the wash solution prepared earlier. Wash by emptying the wells into a suitable container, tapping the inverted plate on absorbent paper, completely filling the wells with wash solution, and vigorously shaking out the contents.
   Notes: *All air bubbles MUST be removed before proceeding to the next step. If air bubbles remain, repeat vigorous striking of the wells onto an absorbent paper towel on a flat surface until eliminated.*

8. Add 0.150 mL of substrate chromogen solution (bottle 6) to each microwell, including the reagent blank microwell. Incubate the plate at room temperature for 20 minutes.
9. Add 0.05 mL of stop solution (bottle 7) to each microwell, including the blank microwell.
10. Gently tap the side of the frame a few times to ensure mixing.
11. Read absorbance at 450 nm using a plate or strip reader according to the manufacturer's instructions. Blank using the first microwell that contains the mixture of substrate chromogen and stop solution (do not blank with air). If your plate or strip reader doesn't automatically blank against the first microwell, calculate the values for the negative control, positive control, and all other sample wells, subtract the absorbance value of the reagent blank (well A1) from the absorbance values registered in all other wells.

INTERPRETATION OF RESULTS

Control values: The absorbance value for the negative control must be $\leq$ 0.15, and absorbance value for the positive control must be $\geq$ 1.00. If either control falls out of the acceptable range, the test is invalid and must be repeated.

Negative criterion: Tests producing absorbance values < 0.10 are negative for the presence of *L. mono* in the test samples.

Positive criterion: Tests producing absorbance values $\geq$ 0.10 are positive for the presence of *L. mono* in the test samples. A positive test result should be confirmed by standard culture procedures.

RECOMMENDED CONFIRMATION PROCEDURE

Neogen recommends that positive results be confirmed culturally by streaking the secondary enrichment culture onto a *Listeria* selective/differential agar plate (MOX agar is recommended), and by continuing with biochemical identification of presumptive *L. mono* isolates using standard procedures.

REFERENCES

1. U. S. Food and Drug Administration. 2003. Detection and enumeration of *Listeria monocytogenes* in foods. Bacteriological Analytical Manual online, chapter 10. http://www.cfsan.fda.gov/~ebam/bam-10.html
2. USDA-FSIS. 2005. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg and environmental samples. Microbiology Laboratory Guidebook, chapter 8. http://www.fsis.usda.gov/Ophs/Microlab/Mlg_8_04.pdf
3. Van Netten, P., Perales, I., van de Housdijk, A., Curtis, G. D., 1989. Liquid and solid selective media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp. Intl. J. Food Microbiology. 8, 299-316

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8 a.m. and 6 p.m. Eastern time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Services. Assistance is available on a 24-hour basis by calling 800/234-5333.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's Web site at www.neogen.com.

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