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SKU #: 700002846
REF #: 9824

NEOGEN®
ANSR®

for Listeria monocytogenes

BY AFNOR CERTIFICATION
NF VALIDATION
EN ISO 16140

NEO 35/04-03/16
Alternative Analytical
Methods For Agribusiness
nf-validation.afnor.org/en

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ANSR® for *Listeria monocytogenes*

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Intended Use

The ANSR® method for *Listeria monocytogenes* provides rapid and accurate detection of *Listeria monocytogenes* in a wide variety of foods and environmental samples.

Neogen® is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing.

ANSR for *Listeria monocytogenes* has not been evaluated with all possible food products, food processes, testing protocols, or with all possible microorganism strains.

Neogen has not documented ANSR for *Listeria monocytogenes* for use in industries other than Food and Beverage industries. For example, Neogen has not documented ANSR for *Listeria monocytogenes* for testing pharmaceuticals or cosmetics.

Neogen's ANSR *Listeria monocytogenes* assay has been certified by NF VALIDATION as an alternative to the reference standard ISO 11290-1, according to ISO 16140-2, for the detection of *Listeria monocytogenes* in all food products for human consumption and in environmental samples. For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate NEO 35/04-03/16 available on the website: nf-validation.afnor.org/en or on request from Neogen.

In an AOAC Research Institute Performance Tested Method℠ Study, ANSR for *Listeria monocytogenes* was found to be an effective procedure for detection of *Listeria monocytogenes* in hot dogs, Mexican-style cheese, cantaloupe, guacamole, pasteurized liquid egg, sprout rinse water, and sponge samples taken from stainless steel surfaces. For AOAC approved methods refer to the 9824 U.S. kit insert.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at www.neogen.com, or contact your local Neogen representative or authorized distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any Neogen product do not constitute a guarantee of the quality of the matrices or processes tested.

Assay Principles

ANSR for *Listeria monocytogenes* is an isothermal, amplified nucleic acid assay. The ANSR for *Listeria monocytogenes* method is based on nicking enzyme amplification reaction (NEAR) technology. Target DNA is amplified through a mechanism of polymerization from the ends of nicks created in double-stranded DNA by the action of a specific endonuclease. Amplified target sequences are detected in real-time using fluorescent molecular beacon probes.

A two-stage lysis reaction is performed, first at 37 ± 2°C for 10 minutes, then at 80 ± 2°C for 20 minutes. Next, a portion of the lysed sample is transferred to a strip tube containing lyophilized ANSR reagents. The tubes are sealed and incubated at 56 ± 1°C on the ANSR reader. Results are generated by the reader and displayed in the ANSR software within 10 minutes. Positive results may be confirmed from the enrichment cultures following standard procedures. Each tube of ANSR reagents contains an internal positive control, ensuring that the reagents are functioning properly.

Intended User

The ANSR for *Listeria monocytogenes* test is designed for use by personnel with appropriate training in microbiology. Training in the use of the ANSR test system is available through Neogen.

Materials Provided

1. 12 strips of 8 cluster tubes, 1.2 mL
2. 12 strips of 8 reaction tubes, 200 µL, containing lyophilized ANSR for *Listeria monocytogenes* reagents in 2 sealed foil pouches with desiccant pack

3. 12 strips of 8 permanent caps for reaction tubes
4. 1 bottle lysis reagent suspension buffer, 60 mL
5. 3 vials containing lyophilized lysis reagents

Equipment Required

1. ANSR reader (Neogen item 9828)
2. Computer and software for connection to ANSR reader (Neogen item 9832)
3. 1 dual heater block with aluminum block inserts for 1.2 mL cluster tubes, 80 ± 2°C and 37 ± 2°C (Neogen items 9386–DUAL48-230 and (2) 9829–48 or equivalent)
4. Pipettor, 20–200 µL (Neogen item 9276 or equivalent)
5. Pipettor, 100–1000 µL (Neogen item 9463 or equivalent)
6. Pipette tip rack, 100–1000 µL, sterile (Neogen item 9487 or equivalent)
7. Pipettor, 10–100 µL, 8-channel (Neogen item 9388 or equivalent)
8. Pipette tips, 100 µL, sterile, filtered (Neogen item 9389 or equivalent)
9. Stomacher or equivalent (optional)
10. Vortex, adjustable speed (Neogen item 9494 or equivalent)
11. 3 thermometers (Neogen item 9518 or equivalent)
12. Timer, 3-channel (Neogen item 9426 or equivalent)
13. Optional-for-use heater block with 0.2 mL reaction tube aluminum block insert, 56 ± 1°C (Neogen items 9386-48D230 and 9829-64 or equivalent)
14. Webcam (Neogen item WEBCAM)
15. ANSR Ethernet cable (Neogen item 9835)
16. 10 mL pipette pump (Neogen item 9277 or equivalent)
17. Pipettes, sterile serological (Neogen item 8686 or equivalent)
18. 40-slot, 20 mm test tube rack, autoclavable (Neogen item 9553 or equivalent)

Other Materials Required

1. Stomacher-type bags for sample enrichment. Filtered bags are recommended (Neogen item 6827)
2. Graduated cylinder, 250 mL (Neogen item 9368)
3. 1 L purified water
4. 20 L RO/deionized water for preparation of the µPREP® LESS Plus Medium
5. µPREP Filter Unit for preparation of the µPREP LESS Plus Medium (Neogen item MPA001)

Media Enrichment Broth Required

1. LESS Plus Medium (Neogen item NCM0202)
2. LESS Plus Medium, 3 L x 3 bags (Neogen item NCM3400)
3. LESS Plus Medium, µPREP Bag (Neogen item NCM3206)

Storage

Store ANSR reagents at 2–8°C. After removing reaction tubes from the foil pouch, promptly reseal the pouch. Leave the desiccant pack in the pouch at all times.

Precautions

1. Use good microbiology laboratory practices, such as ISO 7218.
2. Dispose of used pipette tips in a covered container containing a fresh solution of 10% bleach. The 10% bleach solution should be made fresh each day. Undiluted stock solutions of bleach should be used within 30 days of opening.
3. Discard bleach solution and tips as regular waste at the end of each day.
4. *Listeria monocytogenes* is a known hazard to pregnant women and immunocompromised individuals. Consult your facility safety director for specific instructions.
5. Do not use reagents beyond the expiration date.
6. Use of enrichment media and incubation times or temperatures other than those specified may lead to erroneous results.
7. Remove reaction tubes from the foil pouch just before use and keep covered until heating process begins. Reseal the pouch containing the remaining reaction tubes to avoid prolonged exposure to light. More than 15 minutes of total exposure time may lead to erroneous results.
8. Do not, under any circumstance, remove caps from reaction tubes after the assay has been started. This is essential in order to prevent accidental contamination of the environment with amplification products.

9. Exercise care in all pipetting steps to avoid cross-contamination of samples.
10. Complete all assay steps in sequence, avoiding delays between steps.
11. Tap reaction tubes on bench top to make sure lyophilized reagents are at the bottom of the tube prior to adding lysed sample.
12. The laboratory equipment (pipettes, tubes, etc.) must not circulate from one work station to another.
13. Use powder-free gloves. Change gloves often, especially if you suspect they are contaminated.
14. Clean work spaces periodically with at least 10% bleach and other decontaminating agent.
15. It is strongly advised to work under a hood or a PCR workstation during lyses and amplification steps.
16. The Neogen ANSR *Listeria monocytogenes* kit does not differentiate any one *Listeria monocytogenes* strain from another.
17. To reduce the risks associated with misinterpretation of results, Neogen has not documented this product for use in industries other than food and beverage.
18. Do not use this product for the diagnosis of conditions in humans or animals.

Preparation of Enrichment Broth (LESS Plus Medium)

1. Dissolve 44 g of the medium into one liter of purified water. Heat the solution with frequent agitation to completely dissolve the medium if necessary. Autoclave at 110°C for 15 minutes.

Preparation of Enrichment Broth (µPREP LESS Plus Medium)

1. Reconstitute 20 L bag of µPREP LESS Plus Medium with 20 L of RO/deionized water via µPREP Filter Unit. Do not autoclave.
   Note: See Neogen NCM3206 and MPA001 product information sheets.

Sample Preparation

1. Weigh X g sample in a Stomacher-type bag.
2. Dilute 1:10, X g or X mL of sample in 9 (x) X mL of LESS Plus broth. For example, dilute 25 g or 25 mL of sample in 225 mL of LESS Plus broth.
   Note: For swab testing, the volume of broth should cover the swab sample.
   Note: In the context of NF VALIDATION mark, no samples over 25 g were tested.
   Note: If needed, prepare samples according to standards for the product concerned (ISO 6887 series).
3. Homogenize (Stomacher, etc.).

Sample Enrichment

1. Incubate the culture at 30 ± 1°C for 27 ± 3 hours.
2. To test single samples follow assay procedures.
   Note: Samples can be left at room temperature for 2 hours when carrying out the analysis.
   Note: It is possible to store the enriched LESS Plus broth between +2°C and +8°C for 72 hours maximum, following the last incubation at 30°C.
   Note: For specific matrices, contact Neogen support (out of the scope of NF VALIDATION). For example, for morges testing, we recommend to incubate the culture at 30 ± 1°C during 18–24 hours, pipette 1 mL of the enriched LESS Plus broth and dilute in 9 mL of LESS Plus broth supplemented with 100 µL of Amphotericin B (final concentration of 2.5 µg/mL) and 100 µL of Polymyxin-B (final concentration of 64000 IU/L). Incubate 6 ± 1 hours, then follow the assay procedure.
   Note: Do not shake the suspension before collecting the sample and avoid collecting large fragments of food debris. For food samples with a fatty surface layer, collect the sample from just below this layer.

LYSIS Reagent Solution Preparation

1. Reconstitute 1 vial of lyophilized lysis reagents with 18 mL of lysis reagent suspension buffer by adding the buffer to the reagent vial. Swirl gently to mix.
   Note: 1 vial of lysis reagents is enough for approximately 32 samples. Prepared lysis reagent solution can be stored at 2–8°C for up to 30 days.

ANSR Test Procedure

Prior to starting the assay:

1. Preheat one lysis heater block to 80 ± 2°C. Preheat the second lysis heater block to 37 ± 2°C. If using the optional single heater, preheat to 56 ± 1°C. Use the thermometer for the temperature reading.
2. Remove the foil pouch containing the reaction tubes from the refrigerator and allow the kit to warm at room temperature for 15 minutes. To avoid excess light exposure, leave reaction tubes in foil pouch until they are needed.
   Note: Keep the lysis reagent solution in the refrigerator until ready to use.

3. Connect the ANSR reader to the computer via USB or ethernet and turn the computer on.
4. Turn on the ANSR reader. The reader will preheat to 56 ± 1°C.
5. Start the ANSR software and click the connect button. Input sample IDs, lot number, and user information.
   Note: For instructions on using the reader and software, see the user guide that came with the ANSR reader.
6. ANSR software versions up to 1.8.3 were used in the context of NF VALIDATION. Please check with your technical representative for the latest version.

Assay Procedure

1. Add 50 µL enrichment culture (or dilution) to a 1.2 mL cluster tube(s) using a micro-pipette with 100 µL filtered tips. Ensure that only the tips are in contact with the broth or the stomacher bag and use a new pipette tip for each sample.
   Note: To reduce the risk of cross contamination, it is possible to pipette 1 mL from the enrichment bag into a tube using a regular pipette. Then using a micro-pipette, remove 50 µL from the tube and carry out the assay.
   Note: Cluster tubes may be pulled apart to provide the number of tubes needed.
2. Add 450 µL lysis reagent solution to each cluster tube(s) containing culture.
   Note: Return the lysis reagent solution to the refrigerator after use (within 1 hour).
3. Incubate the cluster tube(s) at 37 ± 2°C for 10 minutes.
4. Immediately transfer the cluster tube(s) to the 80 ± 2°C heater block and incubate for 20 minutes.
   Note: The 80 ± 2°C incubation time may be extended to a total of 60 minutes for the purpose of managing staggered assay start times.
5. For 3–5 minutes before the end of the lysis step, preheat the ANSR reagents to 56 ± 1°C by placing the reaction tube(s) in the ANSR reader.
   Note: The strip of reaction tubes may be cut to provide the number of tubes needed. Keep all unused tubes in the sealed foil pack. Ensure the pellet in the reaction tube(s) is at the bottom by tapping the tubes gently on the bench top.
6. After the completion of the 20–60 minute lysis incubation, remove and discard the caps from the reaction tube(s) in the ANSR reader.
   Important: Proceed with steps 7–9 without delay. The transfer of the sample from the lysis tubes at 80°C to the reaction tubes should be completed within 1 minute.
7. Using an 8-channel pipette and 100 µL filtered tips, carefully transfer 50 µL from the top third of the lysed sample(s) in the cluster tube(s) to the reaction tube(s). Debris may accumulate at the bottom of the lysis tube(s) that will interfere with assay performance. Avoid transfer of debris by aspirating from the top third of the lysis tube(s). Do not prime the pipette tips and do not mix before aspirating. Place the provided permanent cap(s) on the reaction tube(s).
   Caution: Ensure that the pellet is not touched with the pipette when transferring the lysed sample as this can lead to erroneous results.
   Note: Lysed sample may be transferred from the same cluster tube a maximum of 3 times.
8. Remove the strip(s) of tubes from the reader (or 56 ± 1°C heat block if one was used) and vortex briefly (about 2 seconds), then place back into the reader without delay. Close the reader's lid.
   Note: The reader will not provide accurate results if the lid is open. Keep the lid closed at all times while the assay is running. Contamination may occur if the permanent caps are not placed on the reaction tubes and/or if the permanent caps are removed.
9. Click start in the ANSR software to begin the 10 minute assay.
10. Results will be displayed as positive, negative, or invalid once the assay is finished. If the result is invalid, the test must be repeated from the lysed sample held at 80°C or if necessary after a 1:10 dilution of the lysate in lysis buffer prewarmed to 80°C or after a 1:10 dilution of the pre-enriched LESS Plus medium.

Interpretation of Results

Each tube of ANSR reagents contains an internal positive control. A positive control curve will develop in the case of a valid assay. In the case of an invalid result, the positive control curve should be examined and the assay repeated. The sample matrix may be tested for inhibitory effects, please see assay procedure above, step 10, for details. The ANSR software will indicate the test results as positive or negative for the presence of *Listeria moncytogenes* in the enriched sample. In addition, the real-time fluorescence curve generated from the assay can be viewed.

Confirmation

In the context of the NF VALIDATION certified method, all positive ANSR results need to be confirmed in one of two ways:

1. Using standard tests described in the standardized CEN or ISO methods (including the purification step). For the confirmation test, it is necessary to start from the LESS Plus enrichment broth after the full 27 ± 3 hours enrichment at 30°C.

2. Subculturing 0.1 mL of the LESS Plus Broth enrichment broth onto a selective agar plate (RAPID' *L.mono* or Agar *Listeria* according Ottaviani and Agosti (for example, *Listeria* Chromogenic Agar Neogen item NCM1004)). Incubate the plate following the kit instructions. The presence of typical colonies of *Listeria monocytogenes* is sufficient to confirm the presence of *Listeria monocytogenes*.
   Note: In the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

Disposal

Enrichment cultures and used lysis tubes should be disposed of as biohazard waste according to current local/regional/national/industry standards.

Do not remove permanent caps for any reason from the ANSR reaction tubes once the assay has started, even when disposing of them. Reaction tubes can be disposed of as non-biohazardous waste. It is recommended that they be placed in sealable plastic bags and immediately disposed of to protect against accidental opening.

Customer Service

Neogen customer and technical services can be contacted through neogen.com and product training is available by request.

Safety Data Sheets (SDS) Information Available

SDS's are available for all test kits at neogen.com or by calling 800.234.5333 or 517.372.9200.

Terms and Conditions

Neogen's full terms and conditions are available online.

Warranty

Neogen makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

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Molecular Beacon Probes

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NEAR Technology

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