3M

Product Instructions

Molecular Detection Assay 2 - *E. coli* O157 (including H7)

Product Description and Intended Use

The 3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7) is used with the 3M™ Molecular Detection System for the rapid and specific detection of *E. coli* O157 (including H7) in enriched food and feed samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations [1, 2, 3].

The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing environmental, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

As with all test methods, the source, formulation and quality of enrichment medium can influence the results. Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) with Buffered Peptone Water ISO.

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) test kit contains 96 tests, described in Table 1.

Table 1. 3M Molecular Detection Assay Kit Components

Item	Identification	Quantity	Contents	Comments
3M™ Lysis Solution (LS)	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of 3M Lysis Solution per tube	Racked and ready to use
3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tubes	Pink tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Pink caps	96 (12 strips of 8 caps)		Ready to use
3M™ Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is a sterile enrichment medium, e.g., BPW ISO. Do not use water as a Negative Control.

Safety

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7). Retain the safety instructions for future reference.

⚠ **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

NOTICE: Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

⚠ WARNING

Do not use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) in the diagnosis of conditions in humans or animals.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO/IEC 17025[4], or ISO 7218[5].

To reduce the risks associated with a false-negative result leading to the release of contaminated product:

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Use medium pre-warmed to 41.5 ± 1°C. Do not allow the medium to drop below the incubation temperature range during sample preparation.
- Store the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) with food, feed and food process environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) only with surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer (NB) with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 µL of the neutralizing buffer enrichment into the 3M Lysis Solution tubes. 3M™ Sample Handling products which include 3M™ Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

To reduce the risks associated with exposure to chemicals and biohazards:

- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Incubated enrichment media and equipment or surfaces that have come into contact with incubated enrichment media may contain pathogens at levels sufficient to cause risk to human health.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples and associated contaminated waste according to current local/regional/national/industry standards.
- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer). The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

To reduce the risks associated with cross-contamination while preparing the assay:

- Always wear gloves (to protect the user and prevent introduction of nucleases).

To reduce the risks associated with exposure to hot liquids:

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer). The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

NOTICE

To reduce the risks associated with cross-contamination while preparing the assay:

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.

To reduce the risks associated with a false-positive result:

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at **www.3M.com/foodsafety,** or contact your local 3M representative or 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 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) results. Test several samples, representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw versus pasteurized; fresh versus dried, etc.

Limitation of Warranties / Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

Limitation of 3M Liability

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage and Disposal

Store the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60 days.

Do not use 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

The user should complete the 3M Molecular Detection System operator qualification training, as described in the "Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System" document[7].

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

See Section "Specific Instructions for validated methods" for specific requirements:

Table 3 for enrichment protocols according to AOAC® *Official Method of Analysis℠* 2017.01

Table 4 for enrichment protocols according to NF Validation certificate 3M 01/18-05/17

Sample Enrichment

Tables 2, 3 or 4 present guidance for enrichment protocols for food. It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

Foods

1. Pre-warm BPW ISO enrichment medium to 41.5 ± 1°C.
2. Aseptically combine the enrichment medium and sample according to Tables 2, 3 or 4. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize all matrices except leafy produce and fruit, thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 41.5 ± 1°C for the appropriate time according to Tables 2, 3 or 4.

Table 2. General enrichment protocols

Sample Matrix[a]	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hours)
Raw beef including ground/mince and trim	325 g	975 BPW ISO (pre-warmed)	41.5	10-18
Raw meats including raw beef, pork, poultry, lamb, and bison	25 g	225 BPW ISO (pre-warmed)	41.5	8-18
Leafy produce[b]	200 g	450 BPW ISO (pre-warmed)	41.5	18-24
Other foods including fruit[b], vegetables, fruit/vegetable juices, fresh herbs, raw seafood, raw eggs, raw milk, cookie dough, and processed meats	25 g	225 BPW ISO (pre-warmed)	41.5	18-24
Walnuts or nut mixes containing walnuts (this protocol is appropriate for other nuts including pecans, almonds, pistachios, cashews, and chestnuts,	25 g	225 reconstituted non-fat dry milk	41.5	18-24

(a) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.

(b) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.

Specific Instructions for Validated Methods

AOAC® *Official Methods of Analysis*℠ 2017.01

In AOAC *Official Method of Analysis*℠ program, the 3M Molecular Detection Assay 2 – *E. coli* O157 (including H7) was found to be an effective method for the detection of *E. coli* O157:H7. The matrices tested in the study are shown in Table 3.

Table 3. Enrichment protocols using pre-warmed BPW ISO at 41.5 ± 1°C according to AOAC® *Official Methods*℠ 2017.01

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hours)	Homogenized
Raw ground beef (73% lean)	325 g	975	10-18	Manually by hand or by Stomach
Raw bagged spinach[a]	200 g	450	18-24	Gently agitated by hand for 5 minutes, do not homogenize
Fresh sprouts	25 g	225	18-24	Gently agitated by hand for 5 minutes, do not homogenize
Frozen blueberries[a][b]	25 g	225	18-24	Gently agitated by hand for 5 minutes, do not homogenize

(a) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.

(b) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.

NF Validation by AFNOR Certification

3M 01/18-05/17

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en

For more information about end of validity, please refer to NF VALIDATION certificate available on the website mentioned above.

NF VALIDATION Certified method in compliance with ISO 16140-2[8] in comparison to ISO 16654[3]

Scope of the validation: Raw beef meat, raw dairy products, raw fruits and vegetables

Sample preparation: Samples should be prepared according to EN ISO 16654 and EN ISO 6887[6]

Software Version: See certificate

Table 4. Enrichment protocols using pre-warmed BPW ISO at 41.5 ± 1°C according to NF VALIDATION certified method 3M 01/18-05/17

Protocol	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hours)
Raw dairy products, raw fruits and raw vegetables	25 g	225	41.5	18-24
Raw beef meat	25 g	225	41.5	8-24

NOTES:

- Samples larger than 25 g have not been tested in the NF VALIDATION study.
- The recommended protocol interruption points are after enrichment or after sample lysis. Enrichment broth or sample lysate can be stored at 2-8°C for up to 72 hours. After removing the enrichment broth from storage, resume testing from Step 1 in the **Lysis** section. After removing the sample lysate from storage, resume testing from Step 7 in the **Lysis** section. The lysate can also be stored at -20°C.
- Short enrichment protocols are sensitive to incubation conditions and the temperatures specified in the protocol must be followed. The temperature of the waterbath or the incubator where the broths are prewarmed should be verified to ensure the enrichment broth is reaching the required temperature. The total time for sample preparation, including the delay between the end of the medium pre-warming step and the beginning of incubation of the food sample, must not exceed 45 minutes. Using a ventilated incubator during incubation is recommended.

Preparation of the 3M™ Molecular Detection Speed Loader Tray

1. Wet a cloth or disposable towel with a 1-5% (v:v in water) household bleach solution and wipe the 3M Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

Preparation of the 3M™ Molecular Detection Chill Block Insert

Place the 3M Molecular Detection Chill Block Insert directly on the laboratory bench: The 3M Molecular Detection Chill Block Tray is not used. Use the block at ambient laboratory temperature (20-25°C).

Preparation of the 3M™ Molecular Detection Heat Block Insert

Place the 3M Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of 100 ± 1°C.

NOTE: Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C.

Preparation of the 3M™ Molecular Detection Instrument

1. Launch the 3M™ Molecular Detection Software and log in. Contact your 3M Food Safety representative to ensure you have the most updated version of the software.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M™ Molecular Detection System User Manual for details.

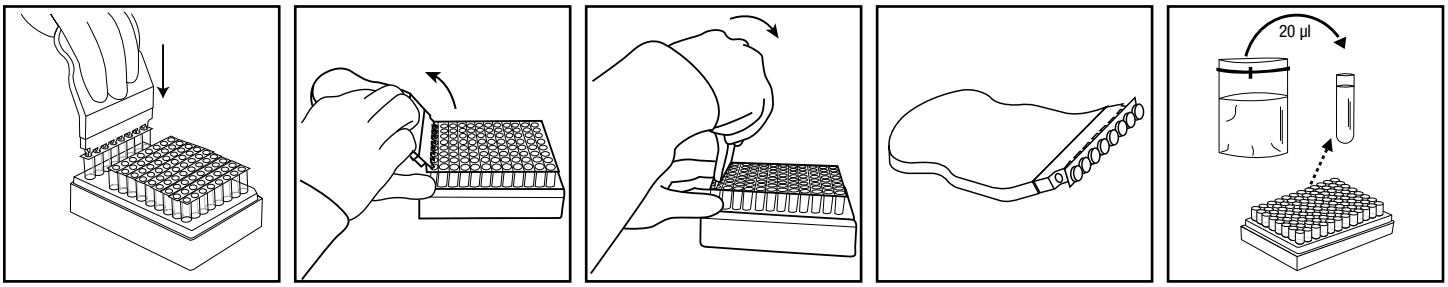
NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

Lysis

1. Allow the 3M Lysis Solution tubes to warm up by setting the rack at room temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the 3M Lysis Solution tubes to room temperature are to set the 3M Lysis Solution tubes on the laboratory bench for at least 2 hours, incubate the 3M Lysis Solution tubes in a 37 ± 1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hours.
3. Remove the enrichment broth from the incubator.
4. One 3M Lysis Solution tube is required for each sample and the Negative Control (NC) sample (sterile enrichment medium).
   - 4.1 3M Lysis Solution tube strips can be cut to desired 3M Lysis Solution tube number. Select the number of individual 3M Lysis Solution tubes or 8-tube strips needed. Place the 3M Lysis Solution tubes in an empty rack.
   - 4.2 To avoid cross-contamination, decap one 3M Lysis Solution tubes strip at a time and use a new pipette tip for each transfer step.
   - 4.3 Transfer enriched sample to 3M Lysis Solution tubes as described below:

     Transfer each enriched sample into an individual 3M Lysis Solution tube **first.** Transfer the NC **last.**

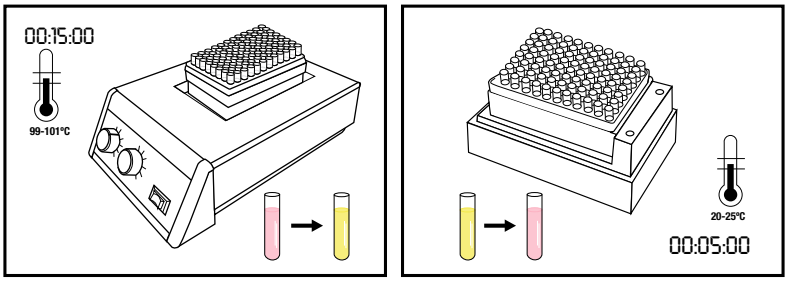
   - 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one 3M Lysis Solution tube strip - one strip at a time.
   - 4.5 Discard the 3M Lysis Solution tube cap – If lysate will be retained for retest, place the caps into a clean container for re-application after lysis.
     - 4.5.1 For processing of retained lysate, see Appendix A.
   - 4.6 Transfer 20 µL of sample into a 3M Lysis Solution tube unless otherwise indicated in the Protocol Tables 2, 3, and 4.



5. Repeat step 4.3 until each individual sample has been added to a corresponding 3M Lysis Solution tube in the strip.
6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW ISO) into a 3M Lysis Solution tube. Do not use water as a NC.
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C.
9. Place the uncovered rack of 3M Lysis Solution tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 1 minutes. During heating, the 3M Lysis solution will change from pink (cool) to yellow (hot).

   Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.
10. Remove the uncovered rack of 3M Lysis Solution tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature without the 3M Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.

11. Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Chill Block Insert.

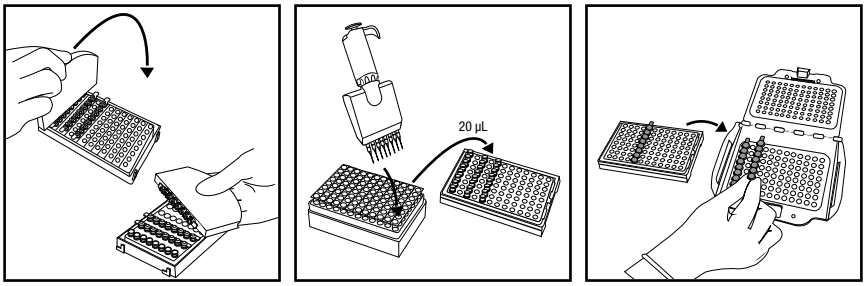


Amplification

1. One 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube is required for each sample and the NC.
   - 1.1 Tube strips can be cut to desired tube number. Select the number of individual 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tubes or 8-tube strips needed.
   - 1.2 Place 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tubes in an empty rack.
   - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select one 3M Reagent Control tube and place in rack.
3. To avoid cross-contamination, decap one 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to a 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube and 3M Reagent Control tube as described below:

Transfer each sample lysate into individual 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tubes **first** followed by the NC. Hydrate the 3M Reagent Control tube **last.**

5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube – one tube strip at a time. Discard cap.
   - **5.1 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the 3M Lysis Solution tube into corresponding** 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) **Reagent Tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.**
   - 5.2 Repeat step 5.1 until individual sample lysate has been added to a corresponding 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube in the strip.
   - 5.3 Cover the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
   - 5.4 Repeat steps 5.1 to 5.3 as needed, for the number of samples to be tested.
   - 5.5 When all sample lysates have been transferred, repeat 5.1 to 5.3 to transfer 20 µL of NC lysate into a 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube.
   - 5.6 Transfer **20 µL of <u>NC lysate</u> into a 3M Reagent Control tube.** Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Then close and latch the lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 60 minutes, although positives may be detected sooner.

10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

> **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes 3M Reagent Control, 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Reagent Tube, and 3M Matrix Control Tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Results and Interpretation

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analysed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation[1,2,3], beginning with transfer from the primary BPW ISO enrichment to secondary enrichment broth(s), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

> **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) amplification reagents have a "background" relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as "Inspect." 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7), non-confirmed by one of the means described above, and in particular for the latex agglutination test), the laboratory must follow the necessary steps to ensure the validity of the results obtained.

Confirmation of Results According to the NF VALIDATION Certified Method

In the context of the NF VALIDATION, all samples identified as positive by the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) must be confirmed by one of the following tests:

Option 1: Using the ISO 16654[3] standard starting from the buffered peptone water[3] enrichment.

Option 2: Implementing a confirmation method consisting of the following: Streak 50 µL of the buffered peptone water[3] enrichment onto a Cefixime Potassium Tellurite Sorbitol MacConkey (CT-SMAC)[3] agar plate. Incubate for 24 ±3 hours at 37°C. Streak characteristic colonies onto nutrient agar and perform latex agglutination test directly onto isolated colonies. If the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) results are not confirmed, perform an immunomagnetic separation step and then streak 50 µL onto CT-SMAC.

Option 3: Using nucleic acid probes as described in the EN ISO 7218[5] standard, performed on isolated colonies (purified or not) from CT-SMAC (see Options 1 or 2). The nucleic acid probes must be different from those used in the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7).

Option 4: Using any other method certified NF VALIDATION, the principle of which must be different from 3M Molecular Detection Assay 2 - *E. coli* O157(including H7). The complete protocol described for this second validated method must be used. All steps prior to the start of confirmation must be common to both methods.

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.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

Appendix A. Protocol Interruption: Storage and re-testing of samples

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see **Lysis** section, 4.5)
2. To store an enriched sample, incubate for a minimum of 18 hours prior to storage.
3. Store at 4 to 8°C for up to 72 hours.
4. Prepare a stored sample for amplification by inverting 2-3 times to mix.
5. Decap the tubes.
6. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ± 1°C for 5 ± 1 minutes.
7. Remove the rack of 3M Lysis Solution tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
8. Continue the protocol at the **Amplification** section detailed above.

References:

1. US Food and Drug Administration Bacteriological Analytical Manual. Chapter 4A: Diarrheagenic *Escherichia coli.* November 2015.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 5.09. Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. Effective Date: 15 January 2015.
3. ISO 16654:2001 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
7. Installation Qualification (IQ)/Operational Qualification (OQ) 3M™ Molecular Detection System. 3M Food Safety.

Explanation of Symbols

www.3M.com/foodsafety/symbols