

Product Instructions

Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*)

Product Description and Intended Use

The 3M™ Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) is used with the 3M™ Molecular Detection System for the rapid and specific detection of Shiga toxin gene (*stx1* and/or *stx2*) and intimin gene (*eae*) from Shiga toxin-producing *E. coli* (STEC, also known as “verocytotoxin-producing *E. coli*”) in enriched foods and food process environmental samples. The term STEC refers to *E. coli* pathotypes capable of producing Shiga toxin type 1 (Stx1), type 2 (Stx2), or both, encoded by *stx1* and *stx2* genes, respectively. STEC containing virulence genes for *stx1* and/or *stx2* and *eae* (intimin gene involved in attaching and effacing phenotype) are designated enterohemorrhagic *E. coli* (EHEC). The screening kit contains two separate reagent tubes, one to detect virulence genes *stx1* and/or *stx2* and the other to detect *eae* from STEC (EHEC). The 3M™ Molecular Detection System Software reports results separately for each of the assays and uses results from both assays to call the sample positive or negative for STEC (EHEC). For a presumptive positive for STEC (EHEC), both gene targets (*stx1* and/or *stx2* and *eae*) must be positive. The *stx* assay does not differentiate between *stx1* and *stx2*, but detects presence of *stx1* and/or *stx2*.

The 3M Molecular Detection Assay uses 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^(1, 2, 3) or as specified by local regulations.

The 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) 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 pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) 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 - STEC Gene Screen (*stx* and *eae*) with buffered peptone water (BPW)-ISO enrichment broth.

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 - STEC Gene Screen (*stx* and *eae*) test kit contains 48 tests of each of *stx* and *eae* reagents, 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 LS per tube	Racked and ready to use
3M™ Molecular Detection Assay 2 -STEC Gene Screen (<i>stx</i>) Reagent Tubes	Orange tubes	48 (2 pouches; containing 3 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
3M™ Molecular Detection Assay 2 -STEC Gene Screen (<i>eae</i>) Reagent Tubes	Red tubes	48 (2 pouches; containing 3 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Orange caps	96 (12 strips of 8 caps)		Ready to use
Extra caps	Red 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

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

A quick start guide is available at www.3M.com/foodsafety

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 - STEC Gene Screen (*stx* and *eae*). 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 - STEC Gene Screen (*stx* and *eae*) 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⁽⁴⁾, ISO/IEC 17025⁽⁵⁾, or ISO 7218⁽⁶⁾.

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.
- Store the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) by the expiration date.
- Use the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) with food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) 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 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 Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, SSL10NB2G, HS10NB, HS10NB2G, and HS2410NB2G.

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 according to current local/regional/national/ regulatory standards.
- 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.

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:

- Change gloves prior to reagent pellet hydration.
- 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.
- 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.

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

- Never open reagent 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.
- Never autoclave reagent tubes post amplification.
- 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, laboratory technique and the sample itself 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 3M Molecular Detection Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) 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 - STEC Gene Screen (*stx* and *eae*) at 2-8°C (35-47°F). 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 (35-47°F) for no longer than 90 days.

Do not use 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) 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 - STEC Gene Screen (*stx* and *eae*) 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.

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.

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

See Section "Specific Instructions for Validated Methods" for specific requirements:

Table 3 for enrichment protocols according to *Performance Tested Method*SM (PTM) Certificate #071902.

Sample Enrichment

Tables 2 and 3 present guidance for general 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. Allow BPW ISO enrichment medium to equilibrate to 41.5 ±1°C.
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Mix all matrices and incubate as outlined in the appropriate protocol table (see Table 2).

Environmental samples

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen Broth. It is recommended to sanitize the area after sampling.



WARNING: Should you select to use neutralizing buffer that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product. Another option is to transfer 10 µL of the neutralizing buffer enrichment into the 3M Lysis Solution tubes.

It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

1. Pre-warm BPW ISO enrichment medium to 41.5 ± 1°C.
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Mix all matrices and incubate as outlined in the appropriate protocol table (see Table 2).

Table 2. General Enrichment Protocols.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL) (pre-warmed)	Enrichment Temperature (± 1°C)	Enrichment Time (hours)	Sample Analysis Volume (µL)
Raw ground beef , pieces and trim ^(a)	375 g	1125 BPW-ISO	41.5	10-18	20
Raw Meat (pork, poultry, lamb, bison) ^(a)	375 g	1125 BPW-ISO	41.5	10-18	20
Leafy Produce ^(b)	200 g	450 BPW-ISO	41.5	18-24	20
Sprouts ^(c)	25 g	225 BPW-ISO	41.5	18-24	20
Raw Dairy ^(d)	25 g or 25 mL	225 BPW-ISO	41.5	18-24	20

^(a) Hand massage the beef (ground beef, pieces and trim) and raw meat (ground pork, poultry and non-beef meat) (b) samples for 30-60 seconds to disperse and break apart clumps after adding BPW-ISO.

^(c) For the leafy produce, rinse enrichment broth (BPW-ISO) over leaves and agitate gently for 30-60 seconds. Do not massage or homogenize leaves.

^(d) For the sprouts, rinse enrichment broth (BPW-ISO) over sprouts for 30-60 seconds and do not massage or homogenize.

^(e) Homogenize the raw dairy samples for 30 to 60 seconds after adding BPW-ISO.

Specific Instructions for Validated Methods

AOAC® Performance Tested MethodSM (PTM) Certificate #071902



In AOAC Research Institute PTMSM studies, the 3M Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*) was found to be an effective method for the detection of STEC. The matrices tested in the study are shown in Table 3.

**Table 3.** Enrichment Protocols According to AOAC PTMSM Certificate #071902.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature ($\pm 1^\circ\text{C}$)	Enrichment Time (hours)	Sample Analysis Volume (μL)
Raw beef trim ^(a)	375 g	1125 BPW-ISO (pre-warmed)	41.5	10-18	20
Raw ground beef ^(a)	375 g	1125 BPW-ISO (pre-warmed)	41.5	10-18	20
Raw Spinach ^(b)	200 g	450 BPW-ISO (pre-warmed)	41.5	18-24	20

^(a) For the beef samples (ground beef and trim) add pre-warmed BPW-ISO to the beef sample. Hand massage for 30-60 seconds to disperse and break apart clumps.

^(b) For raw spinach, add pre-warmed BPW-ISO to the matrix. Rinse liquid over leaves and agitate gently for 30-60 seconds. Do not massage or homogenize leaves.

Preparation of the 3MTM 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 3MTM 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).

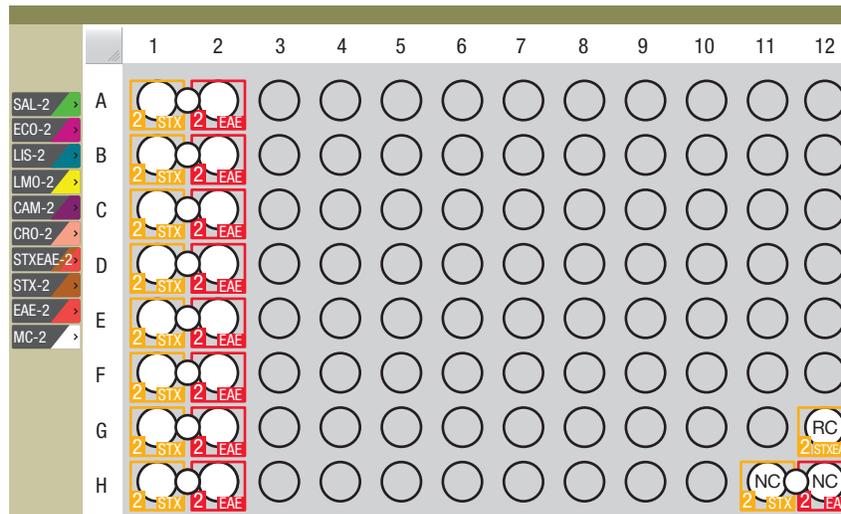
Preparation of the 3MTM Molecular Detection Heat Block Insert

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 $100 \pm 1^\circ\text{C}$.

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 $100 \pm 1^\circ\text{C}$.

Preparation of the 3MTM Molecular Detection Instrument

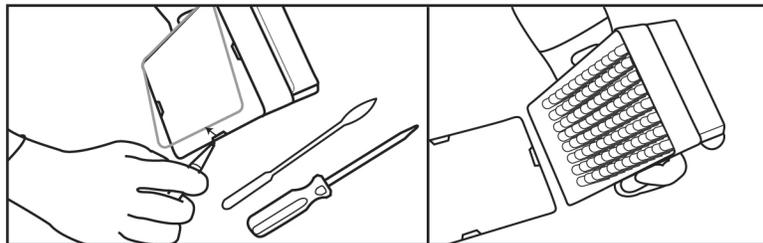
1. Launch the 3M Molecular Detection System Software and log in. Contact your 3M Food Safety representative to ensure you have the most updated version of the software.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.
 - 3.1 Selection of STXEAE-2 icon in software selects two adjacent wells (like A1, A2, B1, B2, etc.), one for *stx* and the other for *eae* reagent tube, as each sample is run with two assays. NC is set up for each of the reagent tube and one RC is set up for the kit.



NOTE: The 3M Molecular Detection Instrument must reach Ready state 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.

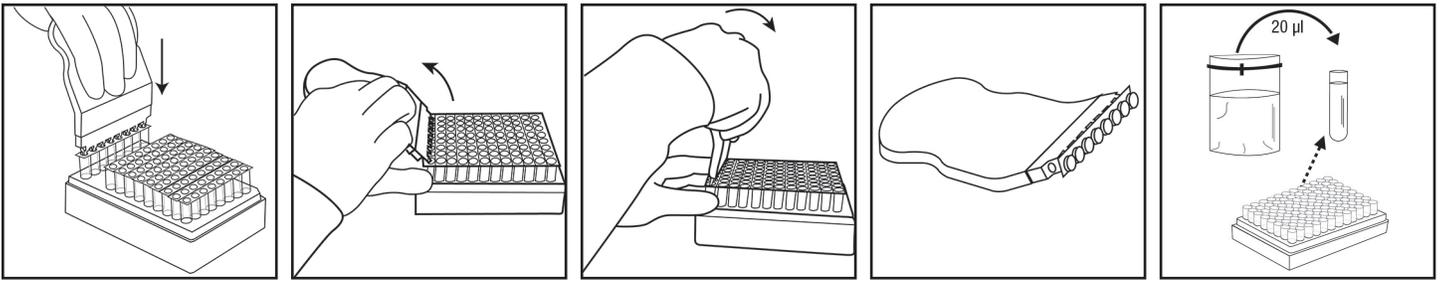
Lysis

Remove the bottom of 3M Lysis Solution Rack with a screwdriver or spatula before placing in in the 3M Molecular Detection Heat Block Insert.

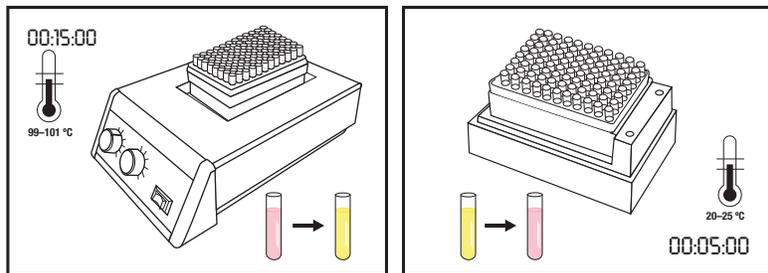


1. Allow the 3M Lysis Solution tubes to warm up by setting the rack at ambient temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the 3M Lysis Solution tubes to ambient temperature are to set the 3M Lysis Solution tubes on the laboratory bench for at least 2 hours, incubate the 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 after inverting.
3. Remove the enriched sample from the incubator.
4. One 3M Lysis Solution tube is required for each sample and the NC (sterile enrichment medium).
 - 4.1. 3M Lysis Solution tube strips can be cut to desired tube number. Select the number of 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 tube 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.

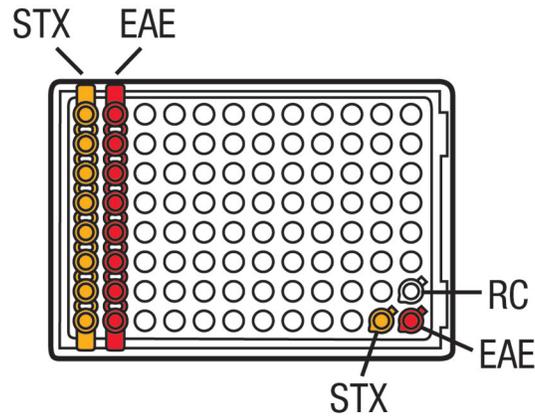


5. Repeat steps 4.4 to 4.6 as needed, for the number of samples to be tested.
6. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW) into 3M Lysis Solution tube. Do not use water as a NC.
7. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at $100 \pm 1^\circ\text{C}$.
8. 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).
 - 8.1. 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.
9. Remove the uncovered rack of 3M Lysis Solution tubes from the 3M Molecular Detection Heat 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 Detection 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 3M Lysis Solution will revert to a pink color.
10. Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Chill Block Insert.



Amplification

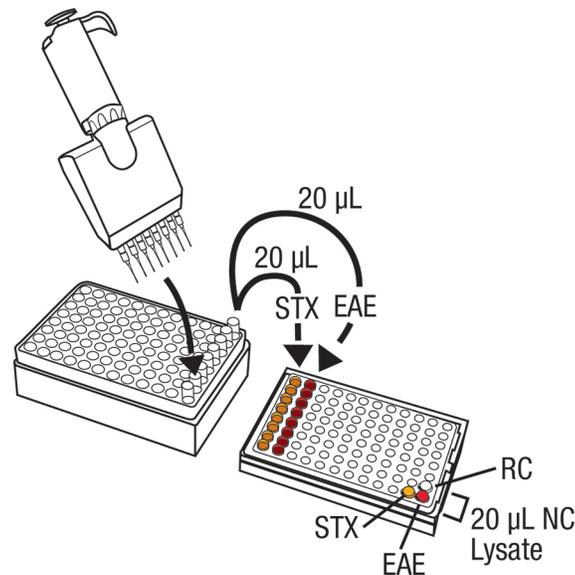
1. One 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and one 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube is required for each sample and the NC.
 - 1.1. Tube strips can be cut to desired tube number. Select the number of individual 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube or 8-tube strips needed.
 - 1.2. Place 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) tubes in an empty rack in one column.
 - 1.3. Place 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) tubes in the adjacent right column.
 - 1.4. Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select one 3M Reagent Control Tube and place in rack.
3. For NC lysate, select one 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) Reagent Tube and one 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube and place in rack.



4. To avoid cross-contamination, decap one 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tube strip at a time and use a new pipette tip for each transfer step.
5. Transfer each of the lysate to a 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tube as described below.
 - 5.1. First, transfer each of the sample lysate to a 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) Reagent Tube as described in 5.5 and 5.6.
 - 5.2. Second, transfer each of the same sample lysate to a 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube in the adjacent right column as described in 5.5 and 5.6.

NOTE: Use new pipette tip for each transfer. Do not use same pipette tip for transfer to *stx* and *eae* reagent tube from the same lysate sample.

- 5.3. After all sample lysate transfer, add NC lysate to each of 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) Reagent Tube and 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube.
- 5.4. Transfer NC lysate last to Reagent Control Tube.



- 5.5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tubes - one strip at a time. Discard cap.
- 5.6. 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 - STEC Gene Screen (*stx* or *eae*) Reagent Tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

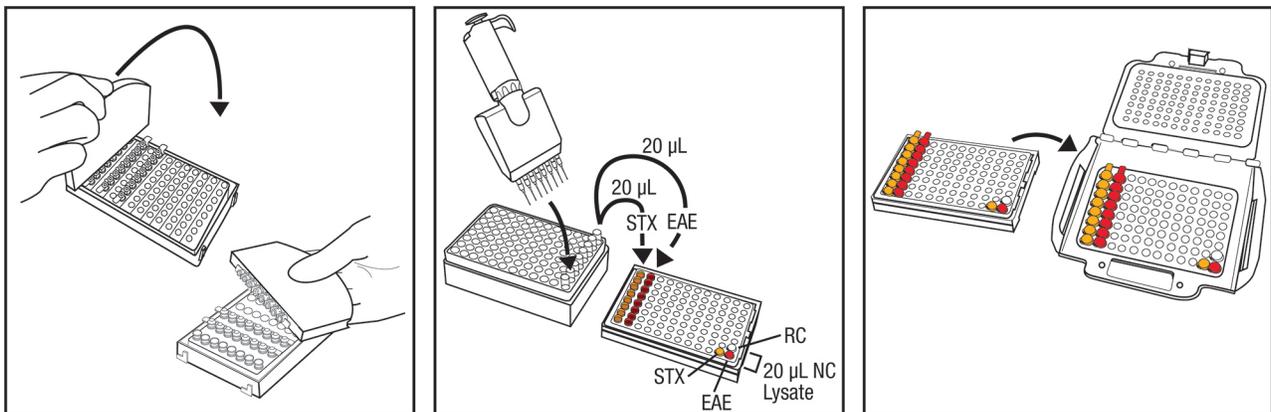
NOTE: Use new pipette tip for each transfer. Do not use same pipette tip for transfer to *stx* and *eae* reagent tube from the same lysate sample.



- 5.7. Repeat step 5.6 until individual sample lysate has been added to a corresponding 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tube in the strip.
- 5.8. Cover the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) 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.
- 5.9. Repeat steps 5.6 to 5.8 as needed, for the number of samples to be tested for both 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) reagent tubes.
- 5.10. When all sample lysates have been transferred, repeat 5.6 to 5.8 to transfer 20 μ L of NC lysate into each of a 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and 3M Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube.
- 5.11. Transfer **20 μ L of NC lysate into a 3M Reagent Control Tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

Transfer each sample lysate into individual 3M Molecular Detection Assay 2 - STEC Gene Screen Reagent Tube (*stx* or *eae*) first followed by the NC. Hydrate the 3M Reagent Control Tube last.

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 System 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 Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) Reagent, 3M Reagent Control, 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.

The 3M Molecular Detection System Software reports results separately for each of the assays (*stx* and *eae*) and uses results from both assays to call the sample positive or negative for STEC (EHEC) (see Figure below). For a presumptive positive for STEC (EHEC), both gene targets (*stx1* and/or *stx2* and *eae*) must be positive. **If any of the individual assays show error or inspect, the final result will be error or inspect. Retest is needed to properly callout the final result**



(see result key). The software also allows to set up individual assays if needed and the sample lysate or fresh lysate from enrichment can be retested with the assays following steps as outlined in Appendix A for retained lysates and steps under Lysis and Amplification section for retained enrichment.

Enriched sample lysate result keys

	Both positive, final result positive
	stx positive, eae negative, final result negative
	stx positive, eae inspect, final result inspect, retest
	stx positive, eae error, final result error, retest
	stx negative, eae positive, final result negative
	stx negative, eae negative, final result negative
	stx negative, eae inspect, final result inspect, retest
	stx negative, eae error, final result error, retest
	stx inspect, eae positive, final result inspect, retest
	stx inspect, eae negative, final result inspect, retest
	stx inspect, eae inspect, final result inspect, retest
	stx inspect, eae error, final result error, retest
	stx error, eae positive, final result error, retest
	stx error, eae negative, final result error, retest
	stx error, eae inspect, final result error, retest
	stx error, eae error, final result error, retest

Negative control keys

	Valid for both, link valid
	Valid for one, other error, link error, retest
	Valid for one, other invalid, link invalid, retest
	Both error, link error, retest
	Both invalid, link invalid, retest
	One error, other invalid, link error, retest

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 enrichment broth to selective plates, confirmation of isolates using appropriate biochemical and serological methods. For matrices specified by MLG 5C, immunomagnetic separation (IMS) should be done prior to plating on selective medium.

NOTE: Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - STEC Gene Screen (stx and eae) 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^(1, 2, 3) or as specified by local regulations.



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

1. To store a heat-treated lysate, re-cap the 3M Lysis Solution 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 $100 \pm 1^\circ\text{C}$ for 5 ± 1 minutes.
7. Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Heat 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. Microbiology Laboratory Guidebook. U. S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory guidebook 5C.00. Detection and isolation of top seven Shiga toxin-producing *Escherichia coli* (STECs) from meat products and carcass and environmental sponges. Feb 4, 2019.
2. US Food and Drug Administration Bacteriological Analytical Manual. Chapter 4A: Diarrheagenic *Escherichia coli*. October 2018.
3. ISO/TS 13136:2012: Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups
4. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good laboratory practice for nonclinical laboratory studies.
5. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
6. ISO 7218. Microbiology of food and animal feeding stuffs - General rules for microbiological examination.
7. 3M Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System. Contact your 3M Food Safety representative to obtain a copy of this document.

Explanation of Symbols

www.3M.com/foodsafety/symbols