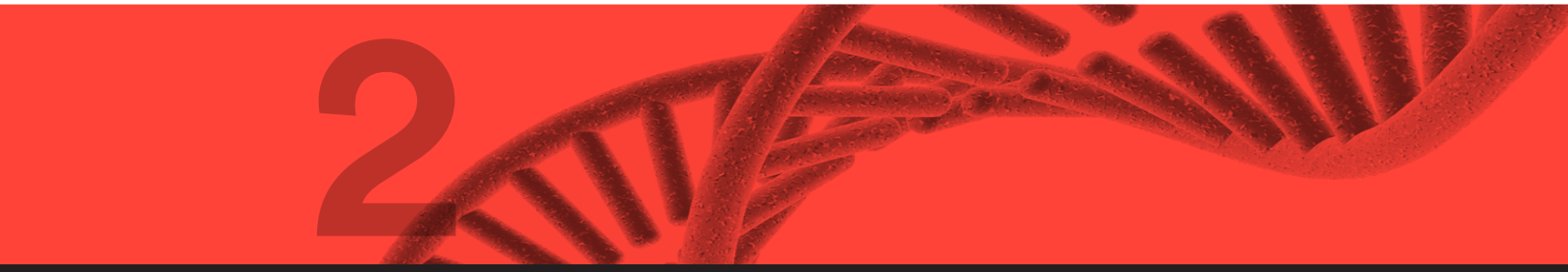
NEOGEN

Product Instructions
MDA2STXEAE48

- (EN) **Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*)**
- (FR) **Kit de détection moléculaire version 2 - STEC Screening des Gènes (*stx* et *eae*)**
- (DE) **Molekulares Detektions Assay 2 - STEC Gene Screen (*stx* und *eae*)**
- (ES) **Sistema de Detección Molecular 2 - Ensayo de Detección Genética de STEC (*stx* y *eae*)**
- (PT) **Ensaio de Detecção Molecular 2 - Teste de Detecção Genética de STEC (*stx* e *eae*)**
- (JA) **病原菌検出アッセイ2 - STEC遺伝子スクリーニング (*stx/eae*) 用**
- (ZH) **分子检测试剂盒 2 - 产志贺毒素大肠埃希氏菌的基因筛选 (*stx* 基因和 *eae* 基因)**
- (TH) **ชุดทดสอบเชื้อระดับโมเลกุล 2 - STEC Gene Screen (*stx* และ *eae*)**
- (KO) **Molecular Detection Assay 2 - 시가독소 생성 대장균 (STEC)(*stx* 및 *eae*)**



STEC Gene Screen (*stx* and *eae*)

Issue Date: 2025-12

NEOGEN®

Product Instructions

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

Product Description and Intended Use

The Neogen® Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) is used with the Neogen® Molecular Detection System for the rapid and specific detection of Shiga toxin gene (*stx*1 and/or *stx*2) 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 *stx*1 and *stx*2 genes, respectively. STEC containing virulence genes for *stx*1 and/or *stx*2 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 *stx*1 and/or *stx*2 and the other to detect *eae* from STEC (EHEC). The Neogen® 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 (*stx*1 and/or *stx*2 and *eae*) must be positive. The *stx* assay does not differentiate between *stx*1 and *stx*2, but detects presence of *stx*1 and/or *stx*2.

The Neogen 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 Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) is intended for use in a laboratory environment by professionals trained in laboratory techniques. Neogen has not documented the use of this product in industries other than food or beverage. For example, Neogen has not documented this product for testing pharmaceutical, cosmetics, clinical or veterinary samples. The Neogen 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. Neogen 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.

Neogen has evaluated the Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) with buffered peptone water (BPW)-ISO enrichment broth.

The Neogen® 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 Neogen Molecular Detection Instrument.

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

The Neogen 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. Neogen Molecular Detection Assay Kit Components.

Item	Identification	Quantity	Contents	Comments
Neogen® 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
Neogen® Molecular Detection Assay 2 - STEC Gene Screen (*stx*) Reagent Tubes	Orange tubes	48 (2 pouches; containing 3 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Neogen® Molecular Detection Assay 2 -STEC Gene Screen (*eae*) 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
Neogen® 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.neogen.com

Safety

The user should read, understand and follow all safety information in the instructions for the Neogen Molecular Detection System and the Neogen 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 Neogen 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 7218:2024[5], or CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL)[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 Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) as indicated on the package and in the product instructions.
- Always use the Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) by the expiration date.
- Use the Neogen 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 Neogen 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 Neogen Lysis Solution tubes. Neogen® Sample Handling Products which include Neutralizing Buffer with aryl sulfonate complex: 700002040 | RS96010NB, 700002011 | RS9604NB 700002003 | SSL10NB, 700002004 | SSL10NB2G, 700002213 | HS10NB, and 700002002 | HS10NB2G.

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 Neogen 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 Neogen® 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 Neogen 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.neogen.com or contact your local Neogen representative or distributor.

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 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 Neogen 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, Neogen has developed the Neogen® Molecular Detection Matrix Control kit. When needed, use the Neogen Molecular Detection Matrix Control (MC) to determine if the matrix has the ability to impact the Neogen 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 Neogen 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, NEOGEN DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any Neogen Food Safety Product is defective, Neogen 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 Neogen within sixty days of discovery of any suspected defects in a product and return it to Neogen. Please contact your Neogen representative or authorized Neogen distributor for any further questions.

Limitation of Neogen Liability

NEOGEN 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 Neogen's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage and Disposal

Store the Neogen 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 Neogen 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 Neogen 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 Neogen Molecular Detection System operator qualification (OQ) training, as described in the "Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for Neogen Molecular Detection System" document[7].

See Section "Specific Instructions for Validated Methods" for specific requirements:
Table 3 for enrichment protocols according to Microval Certificate #2020LR92
Table 4 for enrichment protocols according to *Performance Tested Method*℠ (PTM) Certificate #071902.

Sample Enrichment

Tables 2, 3, and 4 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, 3, or 4).

Environmental Samples

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 Neogen 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.

Sampling Cloth or Mitt

1. For cloth- follow FSIS guidance outlined in the Cloth Sample Collection Method to Replace N60 Excision Sampling for Beef Manufacturing Trimming and Bench[8], by sampling the matrix with the cloth for 90 s, 45 s per side.
2. For mitt- follow the guidance as outlined in USDA Agricultural Research Service No Objection Letter[9] by prewetting the mitt with 25 mL of nBPW, scrub the matrix for 15 s on one side. Flip the mitt over and scrub for additional 15 s. Fold the mitt in half and half again and place it in the sample bag.
3. Allow BPW ISO enrichment medium to equilibrate to 41.5 ±1°C.
4. Aseptically combine the enrichment medium and sample.
5. Mix all samples and incubate as outlined in the appropriate protocol table (see Table 2 or 4).

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
Sampling cloth, sampling mitt[e]	1 cloth or 1 mitt	200 BPW-ISO	41.5	10–24	20

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

[b] For leafy produce, rinse enrichment broth (pre-warmed BPW-ISO) over leaves and agitate gently for 30–60 seconds. Do not massage or homogenize leaves.

[c] For sprouts, rinse enrichment broth (pre-warmed BPW-ISO) over sprouts for 30–60 seconds and do not massage or homogenize.

[d] Homogenize the raw dairy samples for 30–60 seconds after adding pre-warmed BPW-ISO.

[e] Hand massage the samples for 30 seconds after adding pre-warmed BPW-ISO.

Specific Instructions for Validated Methods

MicroVal certificate #2020LR92

The Neogen Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*) has been validated according to ISO 16140-2:2016[10] compared to ISO/TS 13136:2012[3] for the detection of STEC. The matrices tested in the study are shown in Table 3.

For preparation of initial suspensions, follow the instructions of ISO 6887[11] standard. Comply with Good Laboratory Practices according to ISO 7218:2024[5] standard.

Table 3. Enrichment Protocols According to Microval Certificate #2020LR92.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)[a]	Enrichment Temperature (± 1°C)	Enrichment Time (hours)	Sample Analysis Volume (µL)
Raw meat (except poultry)	Up to 375 g	Up to 3375 BPW-ISO (pre-warmed)	41.5	10–18	20
Fresh produce and fruits	Up to 25 g	Up to 225 BPW-ISO (pre-warmed)	41.5	18–24	20

[a] Create a 1:10 matrix to diluent e.g add 3375 mL of prewarmed BPW ISO to 375g sample.

AOAC® *Performance Tested Method℠* (PTM) Certificate #071902

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 071902

In AOAC Research Institute PTM℠ studies, the Neogen 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 4.

Table 4. Enrichment Protocols According to AOAC PTM℠ Certificate #071902.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (± 1°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 ground beef[a]	25 g	225 BPW-ISO (pre-warmed)	41.5	10–18	20
Raw ground pork[a]	375 g	1125 BPW-ISO (pre-warmed)	41.5	10–18	20
Raw poultry parts[a]	375 g	1125 BPW-ISO (pre-warmed)	41.5	10–18	20
Mechanically separated chicken	25 g	225 BPW-ISO (pre-warmed)	41.5	10–24	20
Spinach[b]	200 g	450 BPW-ISO (pre-warmed)	41.5	18–24	20
Sprouts[c]	25 g	225 BPW-ISO (pre-warmed)	41.5	18–24	20
Dried cannabis flower, dried hemp flower[d]	10 g	90 BPW-ISO	41.5	28–32	20
Raw beef, raw pork sampling cloth[e]	1 cloth	200 BPW-ISO (pre-warmed)	41.5	10–24	20
Raw poultry parts, raw poultry carcass sampling mitt[e]	1 mitt	200 BPW-ISO (pre-warmed)	41.5	10–24	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 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.

[c] For the sprouts, rinse enrichment broth (pre-warmed BPW-ISO) over sprouts for 30–60 seconds and do not massage or homogenize.

[d] Dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3%THC).

[e] Hand massage the sample for 30 seconds after adding pre-warmed BPW-ISO.

Preparation of the Neogen® 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 Neogen Molecular Detection Speed Loader Tray.
2. Rinse the Neogen Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the Neogen Molecular Detection Speed Loader Tray dry.
4. Ensure the Neogen Molecular Detection Speed Loader Tray is dry before use.

Preparation of the Neogen® Molecular Detection Chill Block Insert

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

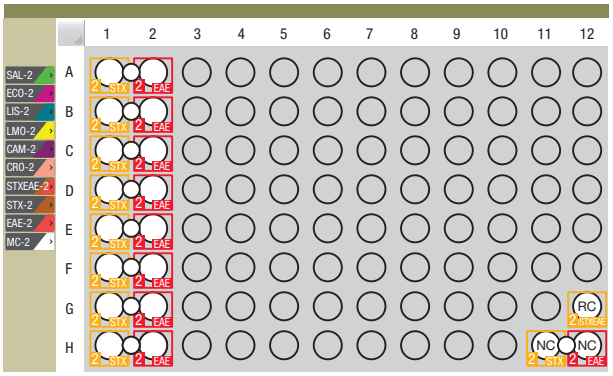
Preparation of the Neogen® Molecular Detection Heat Block Insert

Place the Neogen 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 Neogen 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 Neogen 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 Neogen Molecular Detection Heat Block Insert is at 100 ± 1°C.

Preparation of the Neogen® Molecular Detection Instrument

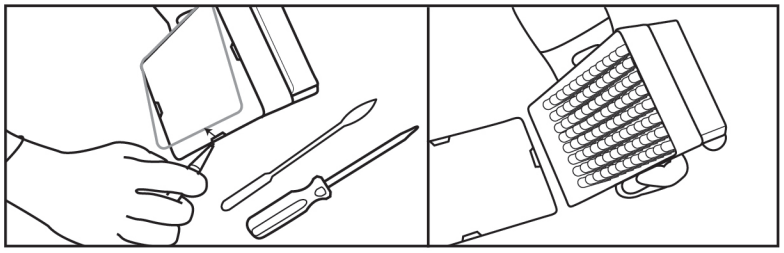
1. Launch the Neogen Molecular Detection System Software and log in. Contact your Neogen Food Safety representative to ensure you have the most updated version of the software.
2. Turn on the Neogen Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the Neogen 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 Neogen Molecular Detection Instrument must reach Ready state before inserting the Neogen 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

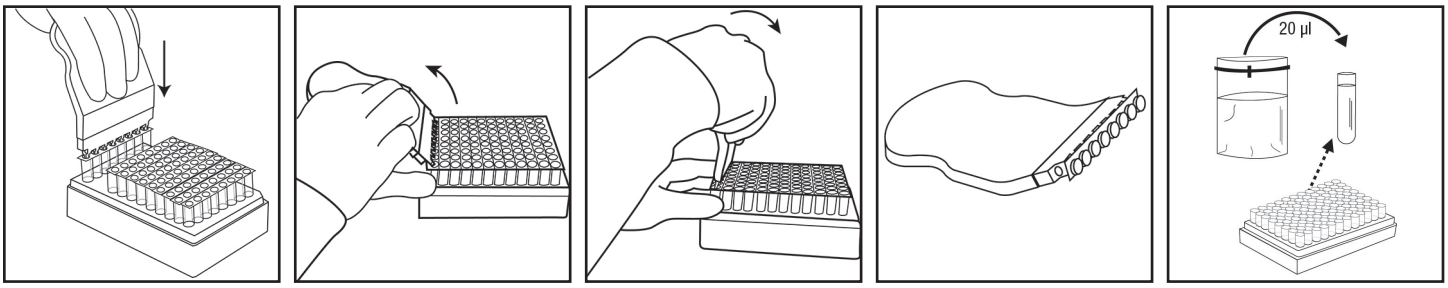
Remove the bottom of Neogen Lysis Solution Rack with a screwdriver or spatula before placing in in the Neogen Molecular Detection Heat Block Insert.



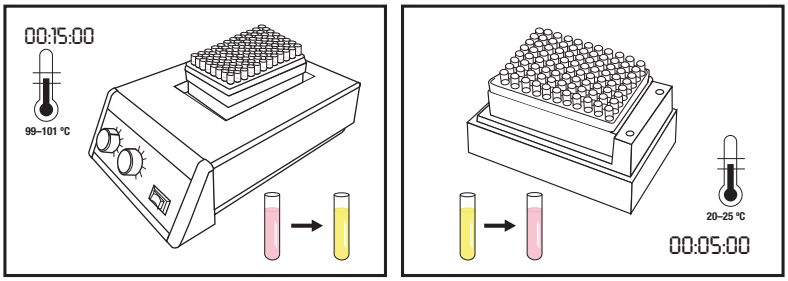
1. Allow the Neogen Lysis Solution tubes to warm up by setting the rack at ambient temperature (20–25°C) overnight (16–18 hours). Alternatives to equilibrate the Neogen Lysis Solution tubes to ambient temperature are to set the Neogen Lysis Solution tubes on the laboratory bench for at least 2 hours, incubate the Neogen 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 Neogen Lysis Solution tube is required for each sample and the NC (sterile enrichment medium).
   - 4.1. Neogen Lysis Solution tube strips can be cut to desired tube number. Select the number of tubes or 8-tube strips needed. Place the Neogen Lysis Solution tubes in an empty rack.
   - 4.2. To avoid cross-contamination, decap one Neogen Lysis Solution tube strip at a time and use a new pipette tip for each transfer step.
   - 4.3. Transfer enriched sample to Neogen Lysis Solution tubes as described below:

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

   - 4.4. Use the Neogen® Molecular Detection Cap/Decap Tool-Lysis to decap one Neogen Lysis Solution tube strip - one strip at a time.
   - 4.5. Discard the Neogen 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 Neogen Lysis Solution tube.

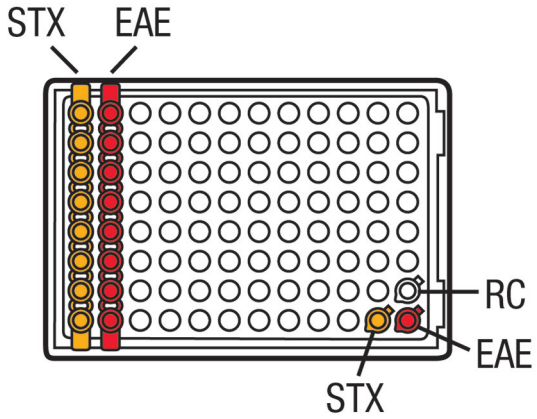


5. Repeat steps 4.4 to 4.6 as needed, for the number of samples to be tested.
6. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW) into Neogen Lysis Solution tube. Do not use water as a NC.
7. Verify that the temperature of the Neogen Molecular Detection Heat Block Insert is at 100 ± 1°C.
8. Place the uncovered rack of Neogen Lysis Solution tubes in the Neogen Molecular Detection Heat Block Insert and heat for 15 ± 1 minutes. During heating, the Neogen 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 Neogen Molecular Detection Instrument.
9. Remove the uncovered rack of Neogen Lysis Solution tubes from the Neogen Molecular Detection Heat Block and allow to cool in the Neogen Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The Neogen Molecular Detection Chill Block Insert, used at ambient temperature without the Neogen® Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the Neogen Lysis Solution will revert to a pink color.
10. Remove the rack of Neogen Lysis Solution tubes from the Neogen Molecular Detection Chill Block Insert.



Amplification

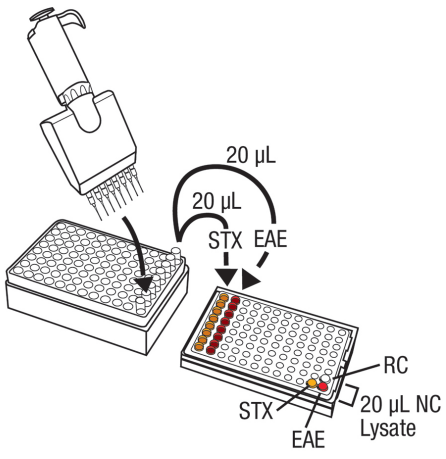
1. One Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and one Neogen Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube is required for each sample and the NC.
   1.1. Tube strips can be cut to desired tube number. Select the number of individual Neogen Molecular Detection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



4. To avoid cross-contamination, decap one Neogen 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 Neogen 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 Neogen 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 Neogen 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 Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx*) Reagent Tube and Neogen Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube.
   5.4. Transfer NC lysate last to Reagent Control Tube.



5.5. Use the Neogen® Molecular Detection Cap/Decap Tool-Reagent to decap the Neogen 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 Neogen Lysis Solution Tube into corresponding Neogen 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 Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tube in the strip.

5.8. Cover the Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* or *eae*) Reagent Tubes with the provided extra caps and use the rounded side of the Neogen Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

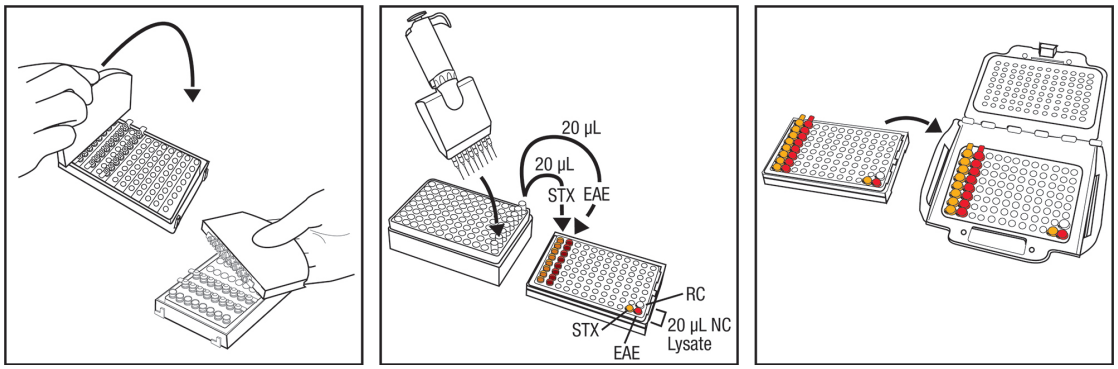
5.9. Repeat steps 5.6 to 5.8 as needed, for the number of samples to be tested for both Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) reagent tubes.

5.10. When all sample lysates have been transferred, repeat 5.6 to 5.8 to transfer 20 µL of NC lysate into each of a Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and Neogen Molecular Detection Assay 2 - STEC Gene Screen (*eae*) Reagent Tube.

5.11. Transfer **20 µL of NC lysate into a Neogen 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 Neogen Molecular Detection Assay 2 - STEC Gene Screen Reagent Tube (*stx* or *eae*) first followed by the NC. Hydrate the Neogen Reagent Control Tube last.

6. Load capped tubes into a clean and decontaminated Neogen Molecular Detection Speed Loader Tray then close and latch the lid.



7. Review and confirm the configured run in the Neogen 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 Neogen Molecular Detection Speed Loader Tray into the Neogen 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 Neogen Molecular Detection Speed Loader Tray from the Neogen 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.

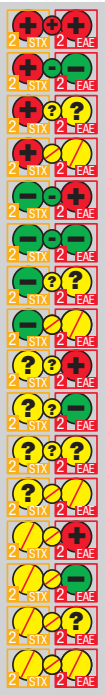
NOTICE: To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) Reagent, Neogen Reagent Control, and Neogen Matrix Control Tubes. Always dispose of sealed 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 Neogen 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 (*stx*1 and/or *stx*2 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 Neogen 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. Neogen 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.

Table 5. Symbols and info for various software results.

Well Type	Well Result Symbol	Result	Interpretation
Sample		Positive	The sample is presumptive positive for the target pathogen.
Sample		Negative	The sample is negative for the target pathogen.
Sample		Inhibited	The sample matrix was inhibitory to the assay. A re-test may be required. Refer to the troubleshooting section and the assay kit Product Instructions for more information.
Sample		Inspect	The presence or absence of the target pathogen was indeterminate. A re-test may be required. Refer to the troubleshooting section and the assay kit Product Instructions for more information.
Sample		Error	No bioluminescence was detected. A re-test may be required. Refer to the troubleshooting section and the assay kit Product Instructions for more information.

Confirmation of Results According to the Microval Certified Method

In the context of the MicroVal validation, all presumptive positive samples by MDA2- STEC Gene Screen (*stx* and *eae*) must be confirmed by following the procedure outlined below:

From the enrichment broth stored at +2°C/+8°C, homogenize by hand and perform a direct streaking. Isolation and confirmation must be initiated within 72 hours following the end of the incubation period.

NOTE: Enrichment broths and lysates can be stored for up to 72 hours at 5°C ± 3°C.

1. Transfer 10 µL of the enrichment broth onto CHROMagar STEC and TBX plates and streak for isolation.
   a. CHROMAgar STEC: Incubate the plate for 18-24 h at 37 ± 1°C.
   b. TBX Agar: Incubate the agar plate for 18-24 h at 37 ± 1°C.

NOTE: Please refer to the appropriate Instruction for Use for any complementary information on the agar plates.

NOTE: If presumptive colonies cannot be isolated, then purify the sample by performing an immuno-concentration step using magnetic beads before confirming on the appropriate isolation agar.

2. Select one to five typical colonies from each plate.
3. Using a single isolated colony, perform a molecular detection of virulence genes as for the screening step (Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx*) or Neogen Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*). Resuspend an isolated colony in 0.5 mL of BPW and proceed to the Molecular Detection Assay Lysis and Amplification steps.

NOTE: Pooling up to 10 colonies is possible to screen for the detection of virulence genes. Positive results from the pooling of colonies will require testing each individual colony to identify the positive isolate(s).

In the event of discrepancies between the MDA2- STEC Gene Screen (*stx* and *eae*) test and confirmation result by using the protocol described above, the laboratory must take the necessary actions to ensure the validity of the results obtained. The recommended protocol is:

1. Subculture the enrichment broth in BPW (0.1 mL + 9 mL of BPW).
2. Incubate the subculture BPW for 4–24 h at 41.5 ± 1°C.
3. Restart the confirmation procedure from step 1 of the Confirmation of results according to the Microval certified method section listed above.

Interpretation of Confirmation Results

A result is considered confirmed positive when the virulence marker(s) (*stx* and *eae*) are detected from an isolated colony on CHROMAgar STEC or TBX agar plates.

Appendix A. Protocol Interruption: Storage and Re-testing of Samples

1. To store a heat-treated lysate, re-cap the Neogen 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 4–8°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 Neogen Molecular Detection Heat Block Insert and heat at 100 ± 1°C for 5 ± 1 minutes.
7. Remove the rack of Neogen Lysis Solution tubes from the Neogen Molecular Detection Heat Block and allow to cool in the Neogen 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 7218:2024. Microbiology of food and animal feeding stuffs - General rules for microbiological examination.
6. CDC Biosafety in Microbiological and Biomedical Laboratories (BMLB) 6th Edition. Effective Date: 29 August 2025.
7. Neogen Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for Neogen Molecular Detection System. Contact your Neogen Food Safety representative to obtain a copy of this document.
8. Cloth Sample Collection Method to Replace N60 Excision Sampling for Beef Manufacturing Trimmings and Bench Trim | Food Safety and Inspection Service https://www.fsis.usda.gov/policy/fsis-notice/05-23.
9. USDA/FSIS Letter of No Objection (NOL) for the MicroTally® Mitt food surface sampler. https://microtally.com/wp-content/uploads/2025/01/Poultry-Mitt-NOL.pdf
10. ISO 16140-2:2016. Microbiology of the food chain – Method Validation – Protocol for the validation of alternative (proprietary) methods against a reference method.
11. ISO 6887 (all parts). Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

Explanation of Symbols

info.neogen.com/symbols

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