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# Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq

NF VALIDATION Certification TRA 02/13-04/22

Part No: 71037-52 (52 tests)

## General Description

Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq (EHEC ID) is an automated nucleic acid amplification system for the genetic identification and confirmation of pathogenic *E. coli* O157:H7 in raw meat, ready-to-cook (RTC) meat products, raw meat poultry, ready-to-cook (RTC) meat poultry product, ready-to-eat (RTE) meat poultry products, ready-to-reheat (RTRH) meat poultry products, raw milk and dairy products, environmental samples. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Based on the workflow described in ISO/TS 13136:2012, the EHEC ID (Lit. No. 20764281) is designed to follow the Assurance® GDS MPX for Top 7 STEC (Lit. No. 20764278) assay to detect the presence of *E. coli* O157:H7. However, the EHEC ID assay does not detect or confirm the presence of Top 6 non-O157 STEC. Follow SECONDARY SCREENING PROTOCOL procedure. Additionally, EHEC ID can be used to confirm isolated colonies from presumptive positive samples for *E. coli* O157:H7. Follow CONFIRMATION OF ISOLATED COLONIES procedure.

## Kit Components

Each Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit contains the following:

- EHEC ID Amplification Tubes
- O157 Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

## Equipment / Materials Required

Other necessary materials not provided include:

- Assurance® GDS Rotor-Gene® thermocycler
- GDS rotor and locking ring
- Laptop computer and software v2.3.103
- PickPen® device and PickPen® tips
- Vortex mixer (IKA® MS3 or equivalent)
- Adhesive film strips
- Sample wells and sample well base
- Resuspension plate
- Stomacher® paddle homogenizer or equivalent
- Stomacher®-type bags with filter or equivalent
- 8-channel micropipette capable of accurately dispensing 30 µL
- Adjustable micropipette capable of accurately dispensing 1.0 mL
- Repeat pipette
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1.0 mL)
- Gel cooling block
- Incubator capable of maintaining 41.5 ± 1 °C
- Incubator capable of maintaining 37 ± 1 °C
- Freezer capable of maintaining -20 ± 5 °C

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Refrigerator capable of maintaining 5 ± 3 °C

# SECONDARY SCREENING PROTOCOL

## Sample Preparation

Please see APPENDIX A for Enrichment Methods Table.

### A. Test Portion Preparation

1. **Raw meat and RTC meat up to 25 g** – Remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C ± 1 °C incubator after a total of 8–26 h of incubation.
2. **Raw beef meat up to 375 g** – Remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C ± 1 °C incubator after a total of 12–20 h of incubation.
3. **Raw meat poultry, RTC, RTRH and RTE meat poultry up to 25 g** – Remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C ± 1 °C incubator after a total of 10-26 h of incubation.
4. **Raw milk and dairy products up to 25 g** – Remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C ± 1 °C incubator after a total of 12–26 h of incubation **or** 20–30 h of incubation (for protocol not following EN ISO 6887 standards (no addition of Tween-80)).
5. **Environmental samples up to 25 g, mL or sampling device** – Aseptically weigh 25 g sweepings or 25 mL process water into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. For environmental monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of mEHEC® media, respectively. Incubate for 12–26 h at 41.5 ± 1 °C.

**Note**: Total enrichment time will be based on the completion of primary Assurance® GDS MPX for Top 7 STEC analysis.

**Note**: Enriched samples can be stored at 5 ± 3 °C for up to 72 h prior to testing with EHEC ID ID **(not applicable for short enrichment protocols)**.

### B. Sample Extraction Protocol

*Change gloves prior to handling reagents.*

1. Vortex **O157 Concentration Reagent**. Immediately transfer 20 µL to each of the required number of GDS sample wells (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover sample wells with adhesive film strips.
2. Transfer 1.0 mL of **Wash Solution** to the required number of GDS sample wells (1 well/sample) using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
3. Transfer 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.
4. Carefully remove the adhesive film from 1 strip of sample wells containing O157 Concentration Reagent. Add 1 mL of presumptive positive enrichment to each sample well. Avoid transferring food particles. A new pipette tip must be used for each enrichment. Cover each strip of sample wells with a new adhesive film prior to adding enrichments to a new strip of wells. **Immediately return samples to incubator for confirmation if necessary.**

5. Place sealed sample wells containing the O157 Concentration Reagent and enrichments on the vortex mixer and vortex at approximately 900 rpm for 5–15 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.
6. Carefully remove and discard the adhesive film from 1 strip of enrichments. Remove the corresponding adhesive film from sample wells containing Wash Solution and from resuspension plate.
7. Load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of enrichments. Stir tips gently for 30 s while continually moving tips up and down from the surface to the bottom of the well. Tap the PickPen® tips against the side of the sample wells to remove excess media droplets.
8. Transfer PickPen® tips to the Wash Solution. With tips submerged, gently stir the PickPen® from side to side for 10–20 s (do not release particles into solution). Gently tap the PickPen® tips against the side of the sample wells to remove excess droplets of Wash Solution.
9. Transfer particles to corresponding row of the prepared resuspension plate. With the tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the buffer. Cover resuspension plate with adhesive film.
10. Repeat steps (6) through (9) for all samples using new tips for each strip of enrichments.

## Test Procedure (Amplification & Detection)

*Change gloves prior to handling reagents*

### A. Preparation of Gel Cooling Blocks

1. Prior to initial use, the gel cooling block must be stored in the freezer (-20 ± 5 °C) for minimum 6 h. When frozen the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -20 ± 5 °C.
2. Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

### B. Preparation of Amplification Tubes

1. The Assurance® GDS Rotor-Gene® set-up and data entry should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes.
2. Remove Amplification Tubes from foil pouch and place them in the frozen gel cooling block. Reseal pouch.
3. Open Amplification Tubes. Using a multi-channel pipette and filter barrier tips, briefly pipette up and down the Resuspension Buffer Tq to mix beads in resuspension plate wells. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube. Firmly press down on each Amplification Tube lid to close. Visually inspect each Amplification Tube to ensure that the cap is securely sealed.
4. Place Amplification Tubes into Assurance® GDS Rotor-Gene® in sequential order, beginning with position #1. Start Rotor-Gene® cycle. Refer to Assurance® GDS user manual for detailed instructions on operating the Rotor-Gene® thermocycler.

   **Note:** The Assurance® GDS Rotor-Gene® must be started within 20 min after addition of the samples to the Amplification Tubes.

## Results

Upon completion of the run, the Assurance® GDS Rotor-Gene® software will provide a results table. Each sample will be identified as **Positive** or **Negative** for *E. coli* O157:H7 or **No Amp** in the Results Tab.

**Positive:** Samples are presumptive positive for *E. coli* O157:H7, meaning they are positive for O157 (*rfbE*) and positive for one or both Shiga toxin genes (*stx1*, *stx2*) and may or may not be positive for H7 (*fliC*).

**Negative:** Samples are negative for *E. coli* O157:H7.

**Note**: If EHEC ID result is negative, and the TOP STEC tab from primary GDS MPX assay was positive, the sample may potentially have *E. coli* O145 and may be confirmed by analysis using Assurance® GDS MPX ID for Top STEC (MPX ID).

**No Amp:** Amplification did not occur. Repeat the test beginning from step **B. Sample Extraction Protocol**. If the No Amp result repeats, contact your local technical service.

| No. | Color | Name | Result | Assay | Kit Lot Number |
|---|---|---|---|---|---|
| 1 | | Sample 1 | Positive | EHEC ID | 1234567 |
| 2 | | Sample 2 | Negative | EHEC ID | 1234567 |
| 3 | | Sample 3 | No Amp | EHEC ID | 1234567 |

# Confirmation

Following 8-30 h enrichment (based on food type analyzed) in mEHEC® at 41.5 °C ± 1 °C, samples can be confirmed from the retained mEHEC® enrichment via the following protocols. In the context of NF VALIDATION, all samples identified as positive by Assurance® GDS EHEC ID for *E. Coli* O157:H7 Tq must be confirmed by one of the following tests:

For dairy products, store mEHEC® broth enrichment at 5 ± 3 °C. Confirmation must proceed from the mEHEC® enrichment. Confirmation cannot proceed from the BHI subculture.

A. *E. coli* O157:H7 may be isolated from *E. coli* O157:H7 positive samples by directly streaking 10 µL of the enrichment to a choice of chromogenic plate: CHROMagar™ O157 plates, Sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC), or EC O157:H7 ChromoSelect Agar Modified. Streak the chromogenic plate for isolation. Incubate plates for 20–24 h at 37 ± 1 °C.

   Confirm up to 5 typical colonies through analysis of isolated colonies by GDS MPX and EHEC ID assays, using protocol CONFIRMATION OF ISOLATED COLONIES FOR E. COLI O157:H7 BY EHEC ID, below.

B. *E. coli* O157:H7 may be isolated from GDS positive samples by plating the GDS O157 STEC concentration reagent which remains in resuspension plate (step C, Test Procedure) to a choice of chromogenic plate: CHROMagar™ O157 plates, CT-SMAC, or EC O157:H7 ChromoSelect Agar Modified. Streak the chromogenic plate for isolation. Incubate plates for 20–24 h at 37 ± 1 °C.

## Isolation of *E. coli* O157:H7 using Concentration Reagent Remainder in Resuspension Plate

### Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Wash Solution
Sterile disposable 10 µL inoculating loops

1. Add 30 µL Wash Solution, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in a new resuspension plate (1 well/sample). Cover resuspension plate wells containing Wash Solution with adhesive film strips.
2. From the sample resuspension plate previously used for EHEC ID analysis (step C, Test Procedure), briefly pipette up and down remainder liquid contained in well to be plated. This will resuspend the IMS beads contained in Resuspension Buffer Tq (approximately 15 µL volume remains).
3. Remove 1 adhesive film strip from Wash Solution resuspension plate from step 1. Transfer 15 µL suspended IMS beads to 1 resuspension plate well containing Top STEC Wash Solution.

4. Briefly pipette up and down the Wash Solution to mix beads in well. Transfer 20 µL from the resuspension plate to first quadrant of the selective plate of choice: CHROMagar™ O157 plates, CT-SMAC, or EC O157:H7 ChromoSelect Agar Modified and streak for isolation on chromogenic plates. Incubate plates for 20–24 h at 37± 1 °C.
5. Confirm up to 5 typical colonies through analysis of isolated colonies by Assurance® GDS MPX for Top 7 STEC and Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq assays, using protocol CONFIRMATION OF ISOLATED COLONIES FOR *E. COLI* O157:H7 BY EHEC ID, below.

   **Note:** The original GDS EHEC ID resuspension buffer plate can be stored at 5 ± 3 °C (refrigeration) for up to 48 h prior to confirmation.

# Confirmation of Isolated Colonies for *E. coli* O157:H7 by EHEC ID

## Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:
Wash Solution
Resuspension Buffer Tq
1 µL disposable inoculating loops, sterile

### Colony Preparation

1. Transfer 500 µL of Wash Solution, using a repeat pipette and a 10 mL pipette tip, to the appropriate number of GDS sample wells (1 well for each suspect colony).

**Note**: The colony tested must be well isolated. If not, re-streak for purity before continuing colony confirmation.

2. Pipet 100 µL of Resuspension Buffer Tq, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in the resuspension plate (1 well / test).
3. Using a 1 µL sterile loop, transfer a small amount of the suspect colony to the sample well containing the Wash Solution.

**Note:** Avoid heavy turbidity for the colony resuspension. It is not necessary to create obvious turbidity in the sample. Mix with the loop for about 5 s and discard the loop into biohazard container.

4. Using a new sterile 1 µL loop, transfer 1 µL of the colony suspension into the prepared resuspension plate well. Stir gently with the loop. Discard the loop into biohazard container.
5. Repeat steps (3) through (4) for up to 5 suspect colonies to be analyzed.
6. Proceed with Assurance® GDS MPX for Top 7 STEC and Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq assays as specified in the directions for use [starting at step **A.** TEST PROCEDURE (AMPLIFICATION & DETECTION)].

### Interpretation and Colony Confirmation Results

Upon completion of the run, the Assurance® GDS Rotor-Gene® software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp**.

**Positive:** An isolate will be considered confirmed positive for *E. coli* O157:H7 if both of the following criteria are obtained:

1. Positive result for EHEC ID: EHEC ID assay identifies serogroup O157 gene (*rfbE*) and either *stx1* and/or *stx2* genes and may or may not be positive for H7 (*fliC*).
2. Positive result for GDS MPX Top 7 STEC: GDS MPX for Top 7 STEC assay provides the *eae* gene results for *E. coli* O157:H7.

**Negative:** Isolate is confirmed negative for *E. coli* O157:H7 if it does not meet the criteria as described above.

**No Amp:** Amplification did not occur. Repeat the test beginning from Confirmation of Isolated Colonies, step **Colony Preparation**. If the No Amp result repeats contact your local technical service.

**Note**: If *E. coli* O157:H7 is not isolated using the direct streak method (above), *E. coli* O157:H7 may then be isolated using either the O157 Concentration Reagent (included within Assurance® GDS EHEC ID for *E. coli* O157:H7 kit) or using the resuspension plate containing remaining IMS particles targeting O157 in resuspension buffer from the GDS positive samples. See steps B and C above.

**Note**: In the event of discordant results (positive presumptive results with the alternative method, non-confirmed by one the means above), the laboratory must follow the necessary steps to ensure validity of the result obtained.

## Storage

Store Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit components at 5 ± 3 °C. Kit expiration is provided on the product box label.

## Precautions

**Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).**

Do not use test kit beyond expiration date on the product box label.

Do not use Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq reagents that have expired.

Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq must be used as described herein.

## Safety

Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit.—This product is not intended for human or veterinary use. Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq must be used as described in the package insert. Contents of the test may be harmful if swallowed or taken internally. The user should read, understand and follow all safety information in the instructions for the Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit. Retain the safety instructions for future reference.

Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state, and federal regulations.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab. Follow all applicable local, state/provincial, and/or national regulations on disposal of amplification tubes. If contamination is suspected, moisten paper towel with bleach solution and wipe all lab benches and equipment surfaces with 10% bleach solution. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution, add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl alcohol solution, add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with 10% bleach solution for a minimum of 15 min or else place the Amplification Tubes into a double bag and dispose outside of the lab.

Assurance® GDS Rotor-Gene.—Improper use of the Assurance® GDS Rotor-Gene may cause personal injuries or damage to the instrument. Some components may pose a risk of personal injury due to excessive heat if improperly handled. For safe use, the instrument must only be operated by qualified laboratory personnel who have been appropriately trained. Servicing of instrument must only be performed by MilliporeSigma Service Engineers.

Sample Enrichment.—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. Always follow standard laboratory safety practices, including wearing appropriate personal protective apparel and eye protection, PPE, 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 industry standards. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state, and federal regulations.

*E. coli* O157:H7 Precautions.—*E. coli* O157:H7 is a biosafety level-3 organism. Biological samples, such as enrichments, have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or

national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes, but is not limited to, protective eyewear, face shield, clothing/laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps. Clean the workstations and laboratory equipment with a disinfectant of choice before and after lab activities (sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

# APPENDIX A – Enrichment Methods

**Table 1. Sample Type and Enrichment Method for *E. coli* O157:H7**

Sample should be taken from the retained enrichment, as described in the Assurance® GDS MPX for Top 7 STEC Directions for Use.

| Food Category | Media | Sample size | Sample:Media Ratio (Media Volume) | Enrichment Time | Enrichment Temperature (media pre-warm) |
|---|---|---|---|---|---|
| Raw meat and RTC meat* | mEHEC® | Up to 25 g | 1:10 (225 mL) | 8–26 h | 41.5 ± 1 °C (41.5 ± 1 °C) |
| Raw beef meat | | Up to 375 g | 1:5 (1500 mL) | 12–20 h | 41.5 ± 1 °C (41.5 ± 1 °C) |
| Raw meat poultry RTC, RTRH and RTE meat poultry* | | Up to 25 g | 1:10 (225 mL) | 10–26 h | 41.5 ± 1 °C (37 ± 1 °C) |
| Raw milk and dairy products* | | Up to 25 g | 1:10 (225 mL) | 12–26 h | 41.5 ± 1 °C (41.5 ± 1 °C) |
| Raw milk and dairy products (except raw milk cheese) | | Up to 25 g | 1:10 (225 mL) | 20–26 h | 41.5 ± 1 °C (41.5 ± 1 °C) |
| Raw milk cheese | | Up to 25 g | 1:10 (225 mL) | 22–30 h | 41.5 ± 1 °C (41.5 ± 1 °C) |
| Environmental samples* | | Up to 25 g or mL or sampling device | 1:10 | 12–26 h | 41.5 ± 1 °C (41.5 ± 1 °C) |

* For these protocols, follow EN ISO 6887 standards.

***NF Validation certificate granted by AFNOR Certification for Assurance® GDS EHEC ID for E. coli O157:H7 Tq as an alternative analytical method for the detection of E. coli O157:H7 in raw meat, ready-to-cook (RTC) meat products, raw meat poultry, ready-to-cook (RTC) meat poultry products, ready-to-eat (RTE) meat poultry products, ready-to-reheat (RTRH) meat poultry products, raw milk and dairy products, and environmental samples in relation to the reference method described in the ISO EN 13136 international standard in accordance with EN ISO 16140-2 (2016). For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate TRA 02/13-04/22 available on the website http://nf-validation.afnor.org/en.***

**TRA 02/13-04/22**
**ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS**

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## Manufacturing Entity

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