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

AOAC® Performance Tested Method 101901
Part No: 71037-52 (52 tests)

General Description

Assurance® GDS, genetic detection system, EHEC ID for *E. coli* O157:H7 Tq (EHEC ID) is an automated nucleic acid amplification system for the genetic confirmation of pathogenic *E. coli* O157:H7 in raw beef, finely textured beef (FTB), and carcass cloths. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Assurance® GDS EHEC ID is designed to follow the Assurance® GDS for *E. coli* O157:H7 Tq assay (No. 71007-100) as a secondary assay. Potential positive samples from the Assurance® GDS for *E. coli* O157:H7 Tq assay can be genetically confirmed with Assurance® GDS EHEC ID to detect *E. coli* O157:H7. Follow SECONDARY SCREENING PROTOCOL procedure. Additionally, Assurance® GDS 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 (Amp) Tubes
- O157 Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

Equipment / Materials Required

Other necessary materials not provided include:

- mEHEC® media
- Assurance® GDS Rotor-Gene®
- 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 repeat pipette
- Adjustable micropipette capable of accurately dispensing 1 mL
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1 mL)
- Gel cooling blocks (2)
- Incubator capable of maintaining 42 ± 1 °C
- Freezer capable of maintaining -20 ± 5 °C
- Refrigerator capable of maintaining 5 ± 3 °C

Note: For this method when a temperature of 42 °C is specified the acceptable temperature range is 41 – 43 °C.

For information on additional materials needed for sample analysis by the Assurance® GDS PickPen®™ PIPETMAX® (PPMX) please see the PPMX User Manual (No. 55240).

AOAC® PERFORMANCE TESTED METHOD #101901

SECONDARY SCREENING PROTOCOL

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Remove enriched sample retained as described in the Assurance® GDS for *E. coli* O157:H7 Tq Directions for Use from 42 °C incubator after a total of 10 – 18 h of incubation (minimum 12 h for frozen FTB).

B. Sample Preparation Protocol

Change gloves prior to handling reagents.

Note: Sample prep can also be completed using the Assurance® GDS PickPen®™ PIPETMAX® (PPMX), for setup procedures please see the Assurance® GDS PPMX User Manual (No. 55240).

a. Vortex O157 Concentration Reagent. Immediately transfer 20 µL to each of the required number of Assurance® GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.

b. Transfer 1.0 mL of Wash Solution to the required number of Assurance® GDS sample wells (1 well/sample) using repeat pipette and 10 mL pipette tips. Cover sample wells with adhesive film strips.

c. 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.

d. Carefully remove adhesive film strip from 1 strip of sample wells. Add 1 mL of potential positive sample to each well containing O157 Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip of wells. Immediately return samples to 42 °C incubator for confirmation if necessary.

e. Place sealed sample wells 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.

f. Carefully remove and discard adhesive film strip from 1 strip of samples. Remove corresponding film strip from sample wells contains Wash Solution.

g. 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 sample wells. Stir 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.

h. Remove adhesive strip from corresponding wells containing Wash Solution. Transfer PickPen®™ tips to the Wash Solution. With tips submerged, gentle stir the PickPen®™ from side to side for 10 – 20 s (do not release particles into solution). Tap the PickPen®™ tips against the side of the wells to remove excess Wash Solution droplets.

i. 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 with adhesive film strip.

j. Repeat steps (g) through (i) for all samples using new tips for each strip of samples.

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents

Note: Amplification tube prep can also be completed using the Assurance® GDS PPMX, for setup procedures please see the PPMX User Manual (No. 55240).

A. Preparation of Gel Cooling Block

a. Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15 °C) for 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 -25 to -15 °C.

b. 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

a. The Assurance® GDS Rotor-Gene® set up and data entry should be completed prior to transferring samples from the resuspension plate into the **EHEC ID Amp Tubes**

b. Remove amplification tubes from foil pouch and place them in the frozen gel cooling block. Reseal pouch.

c. Transfer 30 µL of sample from the resuspension plate wells into each amplification tube using a multi-channel pipette and filter barrier tips. Firmly press down on each amplification tube lid to close. Visually inspect each amplification tube to ensure that the cap is securely sealed.

d. 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®.

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**, **Negative**, or **No Amp**.

Positive: Samples are presumptive positive for *E. coli* O157:H7. This means samples are positive for either "H7" (*fliC*) and/or Shiga Toxin (*stx*1, *stx*2) genes along with being positive for O157 (*rfbE*).

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

No Amp: Amplification did not occur. Repeat the test beginning from step **B. Sample Preparation Protocol**. If the No Amp result repeats, contact Technical Services (BioMTS@milliporesigma.com).

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

Note: Enriched samples can be stored at 2 – 8 °C (refrigeration) for up to 24 h prior to confirmation.

Cultural Confirmation

Samples producing positive results for both Assurance® GDS for *E. coli* O157:H7 Tq and Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq assays should be confirmed from the retained mEHEC enrichment via the following:

(1) Modified U.S. Department of Agriculture (USDA) *Microbiology Laboratory Guidebook* (MLG 5C.00)

(2) Modified U.S. Food and Drug Administrative *Bacteriological Analytical Manual* (BAM Chapter 4A)

(3) *E. coli* O157:H7 may be isolated from EHEC ID positive samples using the GDS O157 concentration reagent and PickPen®™ device.

CONFIRMATION OF ENRICHMENT WITH PICKPEN®™ DEVICE

Equipment / Materials Required

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

O157 Concentration Reagent

Wash Solution

Resuspension plate

Note: Additional O157 Concentration Reagent and wash solution are included in the EHEC ID and GDS *E. coli* O157:H7 kits for performing IMS isolation of *E. coli* O157:H7 with the PickPen®™ device.

a. Aliquot 20 µL, using a 0.5 mL pipette tip and the repeater pipette, of homogenized O157 Concentration Reagent into the Assurance® GDS sample wells (1 well/sample). Cover sample wells with adhesive film strips.

b. Add 1 mL, using a 10 mL pipette tip and the repeater pipette, of Wash Solution into another set of Assurance® GDS sample wells (1 well/sample).

c. Add 100 µL of wash solution, using a 0.5 mL pipette tip and repeater pipette, to the required number of wells in the Resuspension plate. Cover resuspension plate with adhesive film strips.

d. Carefully remove adhesive film strip from 1 strip of sample wells. Following incubation, gently mix enriched presumptive positive samples by hand to ensure homogeneity. Add 1 mL of the enriched sample to each sample well that contains the Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Seal each row of the sample wells with new adhesive film strip.

e. Place the sealed sample wells on the plate vortex mixer at approximately 900 RPM for 5-15 min. **Note:** If necessary adjust the RPM to be certain that liquid does not contact adhesive film.

f. Carefully remove and discard adhesive film strip from 1 strip of samples. Remove corresponding film strip from sample wells contains Wash Solution.

g. 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 sample wells. Stir gently for at least 30 s while continually moving up and down from the surface to the bottom of the wells. Gently tap the PickPen®™ tips against the side of the sample wells to remove excess media droplets.

h. Transfer the PickPen®™ tips to corresponding sample wells containing Wash Solution and gently swirl for at least 10 s.

Note: Do not release particles into solution. Tap the PickPen®™ tips against the side of the wells to remove excess Wash Solution droplets.

i. Transfer PickPen®™ tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen®™ magnets and tap tips gently to release particles into the Wash Buffer.

j. Make additional 1:10 and 1:100 dilutions of the O157 Concentration Reagent (IMS beads) in Wash Solution.

k. Briefly pipette up and down Wash Solution to resuspend beads. Transfer 1:100 and 1:10 diluted beads from the resuspension plate to separate modified Rainbow® agar plates (or other EHEC selective agar) and spread for isolation. Incubate plates for 20–24 h at 35–37°C.

CONFIRMATION OF ISOLATED COLONIES

Equipment / Materials Required

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

1 µL disposable inoculating loops, sterile

Extra Wash Solution

Extra Resuspension Buffer Tq

A. Colony Preparation

a. Transfer 500 µl of Wash Solution to the appropriate number of Assurance® 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.

b. Pipet 100 µL of Resuspension Buffer Tq to the required number of wells in the resuspension plate (1 well / test).

c. Using a 1 µL sterile loop, transfer a small amount of the suspect colony to the sample well containing the Wash Solution.

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

d. 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.

e. Repeat steps (a) through (d) for all suspect colonies to be analyzed.

f. Proceed with Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq assay as specified in the directions for use [starting at step TEST PROCEDURE (AMPLIFICATION & DETECTION), step A. (a)].

B. Interpretation and Colony Confirmation Results

Upon completion of the run each sample will be confirmed and identified as **Positive**, **Negative** or **No Amp.**

Positive: An isolate should be considered confirmed positive for *E. coli* O157:H7 if it possesses O157 gene (*rfbE*) along with either "H7" (*fliC*) and / or Shiga Toxin genes (*stx*1, *stx*2).

Negative: Isolate is confirmed negative if it does not meet the criteria as described above.

No Amp: Amplification did not occur. Repeat the test beginning from prepared resuspension plate, step A. **Colony Preparation, (f)**. If the No Amp result repeats contact Technical Services (BioMTS@milliporesigma.com).

Storage

Store Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit components at 2 – 8 °C. Kit expiration is provided on the product box label.

Precautions

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

Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally. Do not use Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq reagents that have expired.

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 for 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. 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-2 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 work stations and laboratory equipment with a disinfectant of choice before and after lab activities (Sodium hypochlorite solution, phenol solution, Quaternary ammonium solution, etc.).

Manufacturing Entity

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