

Assurance® GDS

Shiga Toxin Genes (O157) Tq

AOAC® *Official Method of Analysis*™ 2005.05

Part No: 71005-100 (100 tests)

General Description

Assurance® GDS for Shiga Toxin Genes (O157) Tq is an automated nucleic acid amplification system for the detection of pathogenic *E. coli* O157:H7/NM in foods, ingredients, carcass cloths, and environmental samples. Assurance® GDS for *E. coli* O157:H7 Tq utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to O157 serogroup prior to genetic analysis for the associated pathogenicity genes. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

EHEC ID is designed as a secondary screening to follow the Assurance® GDS for *E. coli* O157:H7 Tq assay (No. 71007-100, GDS EHEC). Presumptive positive *E. coli* O157:H7 samples from GDS EHEC can be confirmed with EHEC ID. Follow SECONDARY SCREENING PROTOCOL procedure.

Kit Components

Each Assurance® GDS for Shiga Toxin Genes (O157) Tq kit contains the following:

- Shiga Toxin Genes Amplification Tubes Tq
- O157 Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

Equipment / Materials Required

Other necessary materials not provided include:

- mEHEC® media
- Assurance GDS Rotor-Gene® thermocycler
- GDS rotor and locking ring
- Laptop computer and software v.2.3.103
- PickPen® and PickPen® tips
- Vortex mixer (IKA® MS 3, or equivalent)
- Adhesive film strips
- GDS sample wells and sample wells base
- Resuspension plate
- Gel Cooling Block
- Stomacher® paddle homogenizer, or equivalent
- Stomacher®-type bags with filter, or equivalent

8-channel micropipette capable of accurately dispensing 30 µL

Repeat pipette

Adjustable micropipette

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 42 ± 1 °C

Freezer capable of maintaining -20 ± 5 °C

Refrigerator capable of maintaining 5 ± 3 °C

SECONDARY SCREENING PROTOCOL

Sample Preparation

Note: For this method when a temperature of 42 °C is specified the acceptable temperature range is 42 ± 1 °C.

A. Test Portion Preparation and Enrichment

1. Remove enriched sample retained as described in the Assurance® GDS EHEC Directions for Use from 42 °C incubator after a total of 8 – 26 h of incubation.

Note: Total enrichment time will be based on completion of upfront GDS EHEC analysis.

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 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 (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.
4. Carefully remove adhesive film from 1 strip of sample wells containing O157 Concentration Reagent. Mix incubated samples. Add 1.0 mL of presumptive positive sample to each sample well. 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 prior to adding samples to a new strip of wells. **Immediately return samples to incubator for use during confirmation, if necessary.**
5. Place sealed sample wells containing 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 a strip of samples. Remove the corresponding film from sample wells contains Wash Solution.
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 into the first strip of sample wells. Stir gently for 30 s while continually
8. moving tips up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.
9. Remove adhesive film from corresponding wells containing Wash Solution. Transfer PickPen® tips to the Wash Solution. With tips submerged, gentle stir the PickPen® tips from side to side for 10 s (do not release particles into solution). Tap the PickPen® tips against the side of the sample wells to remove excess Wash Solution droplets.

10. Remove the adhesive film from resuspension plate. Transfer particles to corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips.
11. Repeat steps (6) through (9) for all samples.

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents

A. Preparation of Gel Cooling Block

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 upside-down at -20 ± 5 °C.
2. Between each use the gel cooling block should be returned to the freezer and stored upside-down 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 Tq**.
2. Remove Amplification Tubes Tq from foil pouch and place them in the frozen gel cooling block. Reseal pouch.
3. Open Amplification Tubes Tq. Briefly pipette up and down the Resuspension Buffer Tq to resuspend beads. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipettor and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.
4. Place Amplification Tubes Tq into Assurance® GDS Rotor-Gene® in sequential order, beginning with position #1. Start Rotor-Gene® cycle. Refer to Assurance® GDS user manual (No. 55342 / 20516474) 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 Tq.

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**, or **No Amp**.

Positive: Samples are positive for *stx*₁ or *stx*₂ and is confirmed positive for *E. coli* O157:H7.

Negative: Samples are negative for *stx*₁ or *stx*₂ and is confirmed negative for *E. coli* O157:H7.

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

No.	Color	Name	Result	Description	Kit Lot Number
1	■	Sample 1	Positive	Shiga Toxin Genes (O157)	1234567
2	■	Sample 2	Negative	Shiga Toxin Genes (O157)	1234567
3	■	Sample 3	No Amp	Shiga Toxin Genes (O157)	1234567

Confirmation

An aliquot of the mEHEC® enrichment from Assurance® GDS MPX Top 7 STEC positive samples may be culturally confirmed for *E. coli* O157:H7 via USDA-FSIS *Microbiology Laboratory Guidebook (MLG)*, Chapter 5C.01 or via FDA *Bacteriological Analytical Manual (BAM)*, Chapter 4A.

Storage

Store Assurance® GDS for Shiga Toxin Genes (O157) Tq kit components at 5 ± 3 °C. Kit expiration is provided on the product box label.

Precautions

Assurance® GDS for Shiga Toxin Genes (O157) Tq must be used as described herein. Do not use Assurance® GDS for Shiga Toxin Genes (O157) Tq reagents that have expired.

Safety

Assurance® GDS for Shiga Toxin Genes (O157) Tq kit.—This product is not intended for human or veterinary use. Assurance® GDS for Shiga Toxin Genes (O157) 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 Shiga Toxin Genes (O157) Tq kit. Retain the safety instructions for future reference.

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

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.

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/Merck KGaA 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 (e.g., 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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