Assurance® GDS
Shiga Toxin Genes (O157) Tq

AOAC Official Method 2005.05

Part No: 71005-100 (100 tests)

General Description

Assurance® GDS Shiga Toxin Genes (O157) Tq is an automated nucleic acid amplification system for the detection of Shiga Toxin producing *E coli* O157:H7 in foods, ingredients and environmental samples.

Kit Components

Each Assurance® GDS Shiga Toxin Genes for Top STEC 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®
- PickPen® and PickPen tips
- Incubator capable of maintaining 40–43 °C
- Vortex mixer
- Adhesive film
- Sample wells and sample wells base
- Resuspension Plate
- Gel Cooling Block
- Stomacher / Masticator 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)
- Incubator capable of maintaining 40–43 °C

Note: For this method when a temperature of 42 °C is specified the acceptable temperature range is 40–43 °C.

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Remove enriched sample retained as described in the Assurance® GDS system for *E. coli* O157:H7 Tq Directions for Use from 42 °C incubator after a total of 8-18 h of incubation.

B. Sample Preparation Protocol

Change gloves prior to handling reagents.

a. Vortex **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.

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 from 1 strip of sample wells containing Concentration Reagent. Add 1.0 mL of incubated 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 strip prior to adding samples to a new strip of wells. **Immediately return samples to incubator for use during 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 a strip of samples. Remove corresponding film strip from sample wells contains Wash Solution.

g. Load tips onto the PickPen, 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 moving 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 strips from corresponding wells containing Wash Solution. Transfer PickPen to the Wash Solution. With tips submerged, gentle stir the PickPen from side to side for 5–10 s. Tap the PicPen tips against the side of the wells to remove excess Wash Solution droplets.

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

j. Repeat steps (g) through (i) for all samples. Cover resuspension plate with adhesive film strips.

Test Procedure

Change gloves prior to handling reagents

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. Remove Shiga Toxin Genes Amplification Tubes Tq from foil pouch and place them in the frozen gel cooling block. Reseal pouch.

b. 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.

c. 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 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 step **B. Sample preparation Protocol**. If the No Amp result repeats contact BioControl Technical Service.

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

Samples producing positive results for both Assurance® GDS *E. coli* O157:H7 Tq and Assurance® GDS Shiga Toxin Genes (O157) Tq should be confirmed from the retained mEHEC enrichment via USDA-FSIS *Microbiology Laboratory Guidebook* 5A.01 for *E. coli* O157:H7.

Storage

Store Assurance® GDS Shiga Toxin Genes (O157) Tq kit components at 2–8 °C. Kit expiration is provided on the product box label.

Precautions

This product is not intended for human or veterinary use. Assurance® GDS Shiga Toxin Genes (O157) Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

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

Waste may be contaminated with *E. coli* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.
BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803
United States
Sigmaaldrich.com/GDS

Lit. No.: MS_UG4659EN
03/2020