Assurance® *Shiga Toxin* Genes (Top 7) Tq

AOAC Performance Tested Method 071303
Part No: 71018-100 (100 tests)

General Description

Assurance® GDS Shiga Toxin Genes (Top 7) Tq is an automated nucleic acid amplification system for the detection of the *stx*1 and *stx*2 genes in the following select O serogroups of *E. coli*: O103, O111, O121, O145, O26, O45 and O157. Assurance® GDS utilizes a proprietary IMS-based sample preparation procedure to isolate organisms belonging to these specific 7 O serogroups prior to genetic analysis for the presence of the *stx*1 and *stx*2 genes in a variety of foods, including raw ground beef, raw beef trim, raw spinach, and raw mixed greens.

Assurance® GDS Shiga Toxin Genes (Top 7) Tq is designed for use in conjunction with Assurance® GDS Top 7 STEC (*eae*)Tq to detect the presence of Shiga Toxigenic *E. coli* belonging to O serogroups O103, O111, O121, O145, O26, O45 or O157.

Kit Components

Each Assurance® GDS Shiga Toxin Genes (Top 7) Tq kit contains the following:

- Shiga Toxin Gene (Top 7) Amplification Tubes Tq
- Top 7 STEC Concentration Reagent
- Resuspension Buffer Tq
- Top STEC Wash Solution

Equipment / Materials Required

Other necessary materials not provided include:

- mEHEC® media
- Assurance® GDS Rotor-Gene®
- PickPen® and PickPen tips
- Vortex mixer
- Adhesive film strips
- Sample wells and sample wells base
- Resuspension plate
- Stomacher / Masticator 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 41–43 °C

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Beef Samples – Aseptically weigh 375 g test portion into 1,500 mL pre-warmed (41–43 °C) mEHEC media (for 25 g samples, use 225 mL mEHEC). Masticate or homogenize sample by hand for 2 min. Incubate for 10–18 h at 41–43 °C.

b. Fresh Vegetables – Aseptically weigh 375 g test portion into 1,500 mL pre-warmed (41–43 °C) mEHEC media (for 25 g samples, use 225 mL mEHEC). Masticate or homogenize sample by hand for 2 min. Incubate for 10–18 h at 41–43 °C.

Note: Contact BioControl Systems, Inc. for recommended procedures for testing alternate sample types or sizes.

B. Sample Preparation Protocol

Change gloves prior to handling reagents.

a. Vortex Top 7 STEC 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 Top STEC Wash Solution to each of 2 additional sample wells (2 wells/sample) using a 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 Top 7 STEC 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 10–20 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 containing Top STEC 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. Gently tap the PickPen tips against the side of the sample wells to remove excess media droplets.

h. Transfer PickPen to corresponding sample wells containing Top STEC Wash Solution and retract PickPen magnets to release particles into Top STEC Wash Solution.

i. Discard PickPen tips and load a new set of tips onto the PickPen.

j. Extend the PickPen magnets and insert tips into the strip of wells containing the Top STEC Wash Solution and particles. Stir gently for 30 s while continually moving 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 droplets of Top STEC Wash Solution.

k. Transfer PickPen to the second set of sample wells containing fresh Top STEC Wash Solution and gently swirl for 10 s (do not release particles into solution). Tap PickPen tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.

l. 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.

m. Repeat steps (f) through (l) 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. 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.

b. Remove **Shiga Toxin Genes (Top 7) Amplification Tubes Tq** 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 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 positive for *stx*1 or *stx*2 from 1 or more the following O serogroups (O103, O111, O121, O145, O26, O45 and O157).

Negative: Samples are negative for *stx*1 or *stx*2 from the following O serogroups (O103, O111, O121, O145, O26, O45 and O157).

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.	Name	Result	Assay	Kit Lot Number
1	Sample 1	Positive	Shiga Toxin Genes (Top 7)	1234567
2	Sample 2	Negative	Shiga Toxin Genes (Top 7)	1234567
3	Sample 3	No Amp	Shiga Toxin Genes (Top 7)	1234567

Confirmation

An aliquot of the mEHEC enrichment from GDS Shiga Toxin Genes (Top 7) Tq positive samples should be tested with Assurance® GDS Top 7 STEC (*eae*) Tq. Samples which are positive for both the *eae* gene and either *stx*1 or *stx*2 genes, should be considered presumptive positive for Shiga Toxigenic *E. coli* belonging to O serogroups O103, O111, O121, O145, O26, O45 or O157.

Samples producing positive results for both Assurance® GDS Top 7 STEC (*eae*) Tq and Assurance® GDS Shiga Toxin Genes (Top 7) Tq should be confirmed from the retained mEHEC enrichment via USDA-FSIS *Microbiology Laboratory Guidebook* 5A.01. for *E. coli* O157:H7 and via USDA-FSIS *Microbiology Laboratory Guidebook*, 5B.01 for the other top 6 serogroups.

Samples may also be confirmed using Assurance® GDS IMS Panel-Top STEC Kit which contains individual IMS particles for the Top 7 STEC) O serogroups (Part No.61019-100) or Assurance® GDS Poly IMS – Top STEC kit which contains a single mixture of all the Top 7 STEC O serogroups (Part No.61030-100).

Storage

Store Assurance® GDS Shiga Toxin Genes (Top 7) 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 (Top 7) 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

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