Assurance® GDS *E. coli* O157:H7 Tq

MicroVal Certificate No. 2015LR49

Part No: 71007-100 (100 tests)
71007-576 (576 tests)
71007-576ATM (576 tests)

General Description

Assurance® GDS for *E. coli* O157:H7 Tq is an automated nucleic acid amplification system for the detection of pathogenic *E. coli* O157:H7 in raw beef meats, fruits & vegetables, dairy products, and environmental samples. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Kit Components

Each Assurance® GDS for *E. coli* O157:H7 Tq test kit (**100** and **576** tests) contains the following:

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

Each Assurance® GDS for *E. coli* O157:H7 Tq **576ATM** test kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary for **576ATM** kit but sold separately:

61031BC	Wash Solution Kit
34724BC	Resuspension Buffer Tq

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 v2.3.103
- PickPen® device and PickPen® tips
- Vortex mixer (IKA® MS 3, or equivalent)
- Adhesive film strips
- GDS sample wells and sample well base
- Resuspension plate
- Gel cooling block
- Stomacher® paddle homogenizer, or equivalent

Stomacher®-type bags with filter, or equivalent

8-channel micropipette capable of dispensing 30 µL

Repeat pipette

Repeat pipette tips (0.5 mL and 10 mL)

Adjustable micropipette capable of accurately dispensing 20 µL, 45 µL, 0.5 mL, and 1.0 mL

Filter-barrier micropipette tips (50 µL and 1.0 mL)

Incubator capable of maintaining 41.5 ± 1 °C

Incubator capable of maintaining up to 37 ± 1 °C

Freezer capable of maintaining -20 ± 5 °C

Refrigerator capable of maintaining 5 ± 3 °C

Additional materials for the **576** test kit include:

Variable spacing Amplificatiopn Tube holder, 72-well

Variable spacing Amplification Tube holder lid, 72-well

Amplification Tube capping tool

Amplification Tube cap rack, 72-well

Aluminum cooling block, 72-well

72-well rotor and locking ring

MICROVAL CERTIFICATE No. 2015LR49

Approved categories include: Raw beef meats, fruits and vegetables, dairy products, and environmental samples.

TEST PORTION PREPARATION & ENRICHMENT

Note: For this method when a temperature of 41.5 °C is specified, the acceptable temperature range is 41.5 ± 1 °C.

A. Enrichment Media Preparation

1. **Modified EHEC (mEHEC®) broth**

   a. **25 g sample**: Pre - warm 225 mL sterile deionized water at 41.5 ° C overnight. On day of use, aseptically transfer 7.1 g of mEHEC® media into the pre - warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

   b. **375 g sample**: Pre - warm 1500 mL sterile deionized water at 41.5 ° C overnight. On day of use, aseptically transfer 47.3 g of mEHEC® media into the pre - warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

   c. Alternatively, mEHEC® media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder, dispense into desired volume and autoclave at 121 ° C for 15 min. Media must be pre - warmed to 41.5 ° C overnight prior to sample addition.

2. **Brain Heart Infusion (BHI) broth**

   Suspend 37 g of BHI in 1 L of deionized water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

B. Test Portion Preparation and Enrichment – <u>Raw beef meats, fruits and vegetables, and dairy products</u>

Note: For preparations of initial suspensions, follow instructions of EN ISO 16654 and EN ISO 6887 standards, unless stated.

Note: Media must be pre-warmed to 41.5 °C prior to sample addition.

1. **Samples <u>without</u> subculture in BHI**

   a. **Raw beef meats:** Aseptically weigh 375 g test portion into 1500 mL pre-warmed (41.5 °C) mEHEC® media. For 25 g samples, use 225 mL of mEHEC® media. Masticate or homogenize sample.

   Fruits and vegetables: Aseptically weigh 25 g test portion into 225 mL pre-warmed (41.5 °C) mEHEC® media. Masticate or homogenize sample.

   b. Incubate samples for 8 – 14 h at 41.5 °C

   c. Continue to SAMPLE EXTRACTION PROTOCOL.

2. **Samples <u>with</u> subculture in BHI**

   a. **Dairy products:** Aseptically weigh 25 g (mL) test portion into 225 mL pre-warmed (41.5 °C) mEHEC® media. Masticate or homogenize sample.

   b. Incubate samples for 8 – 14 h at 41.5 °C.

   c. Transfer enriched samples to 0.5 mL BHI broth for 2 – 4 h at 37 ± 1 °C as described in D. Sample Extraction Protocol, Step 2.

   d. Continue to SAMPLE EXTRACTION PROTOCOL.

C. Test Portion Preparation & Enrichment – <u>Environmental Samples</u>

1. **Samples without subculture in BHI**

   a. **Environmental monitoring** (stick swabs and sponges): Pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E Broth or Letheen Broth. After collecting sample from surface, add sponge or swab to 100 mL or 10 mL of mEHEC® media, respectively. Incubate samples for 8 – 14 h at 41.5 °C.

   Areas of sampling:

   - Food (and non-food) product contact surfaces, work surfaces and adjacent areas (e.g., blenders, worktables, drip shields, housing)
   - Non-food contact surfaces not close to food product work surfaces (e.g., drains, floors, walls, wheels of cart)

   Note: Sponges and swabs hydrated with Neutralizing Buffer should not be used with Assurance® GDS kits as they may interfere with the PCR reaction.

   b. **Dusts and process water**: Aseptically weigh 25 g sweepings to 225 mL pre-warmed (41.5 °C) mEHEC® media. Aseptically add 25 mL process water to 225 mL of pre-warmed (41.5 °C) mEHEC® media. Incubate samples for 8 – 14 h at 41.5 °C.

D. 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 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.

   For **dairy products**, dispense 0.5 mL of sterile BHI broth to GDS sample wells (1 well/sample) instead of Wash Solution. 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 strip from 1 strip of sample wells containing O157 Concentration Reagent. Following incubation, gently mix enriched presumptive positive samples by hand to ensure homogeneity. Add 1.0 mL of incubated sample to each sample well using adjustable micropipette and 1.0 mL filter barrier tips. 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 confirmation, if necessary.**

5. Place sealed sample wells containing O157 Concentration Reagent and samples on the vortex mixer and vortex at 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 adhesive film from 1 strip of samples. Remove corresponding adhesive film from a strip of sample wells containing either Wash Solution or BHI (for **dairy products**).
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 sample wells. Stir tips 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.
8. For all samples except **dairy products**, transfer PickPen® tips to corresponding sample wells containing Wash Solution. With tips submerged, gently swirl the PickPen® device from side to side for 10 s (do not release partially into solution). Tap the PickPen® tips against the side of the wells to remove excess Wash Solution droplets.
9. Remove adhesive film strip from resuspension plate. 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 Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step 11.
10. For **dairy products:**
    a. Remove adhesive film strip from GDS wells containing BHI. Transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen magnets and tap tips gently to release particles into the BHI. Cover each strip of BHI containing Concentration with a new adhesive film prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 37 ± 1 °C.
    b. Remove adhesive film strip from resuspension plate. Following incubation, transfer the particles from the BHI sample wells to the corresponding column of the prepared resuspension plate using the PickPen® device, as indicated in steps D7 – D9. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate column with adhesive film and continue with step 11.
11. Repeat steps D6 – D10 for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

1. Prior to initial use for **100-** and **576ATM** test kits, the gel cooling block must be stored upside-down in the freezer (-20 ± 5 °C) for 6 h. The gel cooling block will be used to hold the prepared Amplification Tubes Tq. 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 until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.
3. The 72-well aluminum cooling block is for use with the **576** test kit. The aluminum cooling block and should be stored in the refrigerator (5 ± 3 °C).

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 from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for **576** test kit). Reseal pouch.
3. For the **100** and **576ATM** kits, open Amplification Tubes Tq. Briefly pipette up and down resuspension solution to resuspend beads. 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.

4. For the **576** test kit, place tubes into variable-spacing Amplification Tubes holder. Using Amplification Tubes capping tool, remove lids from Amplification Tubes. Place the lid device on top of the holder. Use the Amplification Tube holder blades to slice apart the Amplification Tubes. Separate fully the Amplification Tubes with the variable-spacing holder. Briefly pipette up and down resuspension solution 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. Remove the lid from the holder. Push together the Amplification Tubes with the holder. Cap Amplification Tubes using the Amplification Tubes capping tool. Visually inspect each tube to ensure that the cap is securely sealed.
5. Place Amplification Tubes into Assurance® Rotor-Gene® in sequential order, beginning with position #1. For the **100** and the **576ATM** test kits, use the 36-well rotor and locking ring; for the **576** test kit, use the 72-well rotor and locking ring.

Note: For **576** test kit, after loading Amplification Tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note **2060 / MK_AN4551EN / MS_AN4551EN** for details.

6. 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.

Note: Enriched samples can be stored at 5 ± 3 °C (refrigeration) for up to 72 h prior to testing with Assurance® GDS for *E. coli* O157:H7 Tq.

Results

Upon completion of the run, the Assurance® GDS 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

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

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

No.	Color	Name	Result	Assay	Kit Lot Number
1	■	Sample 1	Positive	*E. coli* O157:H7 Tq	1234567
2	■	Sample 2	Negative	*E. coli* O157:H7 Tq	1234567
3	■	Sample 3	No Amp	*E. coli* O157:H7 Tq	1234567

Confirmation

Following 8 – 18 h enrichment in mEHEC® at 41.5 °C, samples can be confirmed from the retained mEHEC® enrichment. Samples can be held at 5 ± 3 °C for up to 72 h prior to confirmation. For dairy products, store mEHEC® broth (and not BHI subculture) enrichment at 5 ± 3 °C. Confirm samples by ISO method (1) or alternative confirmation (2).

(1) Confirm typical colonies via ISO 16654 (2001/Amd 2:2023): Horizontal method for the detection of *E. coli* O157:H7. Concentrate using immunomagentic particles. Inoculate 50 µL onto Sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC) medium (Merck #1002130500 + #77981 supplement, or equivalent) and second isolation medium. Incubate plates for 21 ± 3 h at 37 ± 1 °C and recommended temperature and specified time, respectively.

(2) *E. coli* O157:H7 may be isolated from EHEC positive samples by directly streaking enrichment to a choice of plate type: CHROMagar™ O157 (Fisher # NC9214592) plates, CT-SMAC plates (Merck #1002130500 + #77981 supplement, or equivalent), or EC O157:H7 ChromoSelect Agar, Modified (Merck # 92587) plates. Streak plate for isolation. Incubate plates for 20–24 h at 36 ± 1 °C.

Confirm typical colonies by aggultination test for O157, either Abraxis *E. coli* O157:H7 (Eurofins # 543070) or Thermo Scientific™ *E. coli* O157 (Thermo # DR0620M) latex test kits.

Storage

Store Assurance® GDS for *E. coli* O157:H7 Tq kit components at 5 ± 3 °C.

Precautions

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

Assurance® GDS for *E. coli* O157:H7 Tq must be used as described herein. Do not use test kit beyond expiration date on the product box label.

Safety

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

Contents of the test may be harmful if swallowed or taken internally.

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 10% bleach solution and wipe all lab benches and equipment surfaces. 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/provincial, 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 (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 (e.g., sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

APPENDIX A – *E. coli* O157:H7 Enrichment Methods

Table 1: Sample Type and Enrichment Method for *E. coli* O157:H7 (Raw beef meats, fruits and vegetables, dairy products)

Food Type	Media	Sample Size	Sample:Total Volume Ratio	Enrichment Time	Incubation Temperature
No BHI subculture					
Raw beef meats	mEHEC	10 –25 g	1:10	8 – 14 h	41.5 ± 1 °C
		25 – 375 g	1:5	8 – 14 h	41.5 ± 1 °C
Fruit and vegetables	mEHEC	10 – 25 g	1:10	8 – 14 h	41.5 ± 1 °C
With BHI subculture					
Dairy Products	mEHEC	10 – 25 g	1:10	8 – 14 h	41.5 ± 1 °C

Table 2: Sample Type and Enrichment Method for *E. coli* O157:H7 (Environmental monitoring)

Environmental Sample	Media	Sample Size	Sample: Total Volume Ratio	Enrichment Time	Incubation Temperature
Environmental samples (Nearby food contact surfaces*) (Distant non-food contact surfaces**)	mEHEC	Swab Sponge	10 mL 100 mL	8 – 14 h	41.5 ± 1 °C
Process water	mEHEC	25 mL	1:10	8 – 14 h	41.5 ± 1 °C
Dust, sweepings	mEHEC	25 g	1:10	8 – 14 h	41.5 ± 1 °C

*Food (and non-food) product contact surfaces, work surfaces and adjacent areas (e.g., blenders, worktables, drip shields, housing)

**Non-food contact surfaces not close to work surfaces (e.g., drains, floors, walls, wheels on cart)

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

Tel: 425-586-3300 www.sigmaaldrich.com

BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

Lit. No. MK_UG5694EN v2.0
04/2025
20516476 v4.0
55347