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, genetic detection system, 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.

Kit Components

Each Assurance® GDS for *E. coli* O157:H7 Tq test kit 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 but sold separately:

61031-100	Wash Solution Kit
34724-100C	Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- mEHEC® media
- Assurance® GDS Rotor-Gene®
- PickPen® device and PickPen® tips
- Vortex mixer
- Adhesive film strips
- Sample wells and sample well base
- Resuspension plateGel cooling block
- Stomacher / Masticator or equivalent
- 8-channel micropipette capable of 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 41.5 ± 1 °C

Additional materials for the 576 test kit include:

Variable Spacing Multi-Channel Pipette

Aluminum cooling block, 72 well

72-well rotor and locking ring

Sample Preparation

A. Enrichment Media Preparation

1. For 25 g sample, pre-warm 225 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 7.1 g of BioControl mEHEC media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.
2. For 375 g sample, pre-warm 1500 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 47.3 g of BioControl mEHEC media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.
3. 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 ± 1 °C overnight prior to sample addition.

B. Test Portion Preparation and Enrichment

For preparations of initial suspensions, follow instructions of EN ISO 6579 and of EN ISO 6887 standards

1. **Raw Beef Meat Samples** – Aseptically weigh 375 g test portion into 1,500 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. For 25 g samples, use 225 mL of mEHEC media. Masticate or homogenize sample. Incubate samples for 8 – 14 h at 41.5 ± 1 °C.
2. **Fruits and Vegetables** – Aseptically weigh 25 g test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC media. Masticate or homogenize sample. Incubate for 8 – 14 h at 41.5 ± 1 °C.
3. **Dairy Products** – Aseptically weigh 25 g test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC media. Masticate or homogenize sample. Incubate for 8 – 14 h 41.5 ± 1 °C. IMS transfer to 0.5 mL Brain Heart Infusion (BHI) for 2 – 4 h at 37 ± 1 °C as indicated in step C(2) below.
4. **Environmental Samples** – Aseptically weigh 25 g sweepings or 25 mL process water into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC media. For sponges, use 100 mL of mEHEC media. For swabs, use 10 mL of mEHEC media. Masticate or homogenize sample. For **environmental** monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of BPW, respectively. Incubate for 8 – 14 h at 41.5 ± 1 °C.

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

C. Sample Preparation Protocol

Change gloves prior to handling reagents.

1. 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.
2. Transfer 1 mL of **Wash Solution** to additional sample wells (1 well/sample) using a repeat pipette and 10 mL tip. Cover sample wells with adhesive film strips.

   For **dairy products**, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well/sample) in place of Wash Solution. Cover sample wells with adhesive film strips.

3. Add 45 µL of **Resuspension Buffer Tq** to the wells in the resuspension plate 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. 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. **Store an aliquot of the enrichment broth at 2 – 8 °C for confirmation step, if necessary.**
5. Place sealed sample wells containing O157 Concentration Reagent and sample 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 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. Transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release partially into solution). 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 C(9).

   For **dairy products**, 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 BHI strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 37 ± 1 °C.
9. Following incubation, transfer the particles from the BHI sample wells to the corresponding row of the prepared resuspension plate using the PickPen® device, steps C(6) – C(9). 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 C(10).
10. Repeat steps C(6) – C(9) for all samples using new tips for each strip of samples.

Test Procedure

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 in the freezer (-25 to -15 °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 at -25 to -15 °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 (2 – 8 °C). To use, place the aluminum cooling block on top of the gel cooling block.

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 (aluminum cooling block for **576** test kit). Reseal pouch.

3. Open Amplification Tubes. 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.
4. 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 FRMMK.2060 for details.

5. 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.

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

No Amp: Amplification did not occur. Repeat the test beginning from step C. Sample Preparation Protocol. If No Amp result repeats, contact BioControl Systems Technical Service.

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

Note: Enriched samples can be stored at 2 – 8 °C (Refrigeration) for up to 72 h prior to testing with Assurance® GDS for *E. coli* O157:H7 Tq.

Confirmation

For confirmation of positive PCR results, proceed as described in Sample Preparation Protocol, except adding 35 µL of Wash Solution in place of the 45 µL of Resuspension Buffer in resuspension plate.

Note: For **dairy products**, no BHI subculture should be performed, instead IMS process as non-dairy samples.

Mix suspended IMS beads well by pipetting up and down. Streak 10 µL suspended beads from resuspension plate onto CT-SMAC agar plate. Incubate plates at 36 ± 1 °C for 24 h. Confirm typical colonies by Latex test (O157 and H7) after purification step on a non-selective agar plate or else use the confirmatory tests described in the ISO 16654:2001.

Storage

Store Assurance® GDS for *E. coli* O157:H7 Tq kit components at 2 – 8 °C. Kit expiration is provided on the product box label.
Do not use Assurance® GDS for *E. coli* O157:H7 Tq reagents that have expired.

Precautions

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

This product is not intended for human or veterinary use. Assurance® GDS *E. coli* O157:H7 Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

If possible, maintain separate work zones and dedicated equipment and supplies for sample preparation and amplification and detection. 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 in 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* O157:H7 which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Manufacturing Entity

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