Assurance® GDS *Salmonella* HET Tq

Part No: 71022-100 (100 tests)
71022-576ATM (576 tests)

General Description

Assurance® GDS, genetic detection system, for *Salmonella* HET Tq is an automated nucleic acid amplification system for the detection of *Salmonella* spp., *S.* Heidelberg, *S.* Enteritidis and/or *S.* Typhimurium (HET) in environmental boot swabs.

Kit Components

Each Assurance GDS for *Salmonella* HET Tq 100 kit contains the following:

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

Each Assurance GDS for *Salmonella* HET Tq 576ATM kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary but sold separately:

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

Equipment / Materials Required

Other necessary materials not provided include:

- Media per Appendix A
- Assurance GDS Rotor-Gene®
- PickPen® device and PickPen tips
- Vortex mixer
- Adhesive film strips
- Sample wells and sample well 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 35–37 °C

Sample Preparation

A. Test Portion Preparation & Enrichment

For **boot swabs**, collect environmental surface samples with a boot swab hydrated with D/E (Dey/ Engley) Broth, Letheen Broth or evaporated (double strength) skim milk. Place 2 boot swabs into 18 oz sample bag. Add 200 mL of Tetrathionate Broth with novobiocin (TT + n) (Appendix A) and homogenize or mix well. Incubate samples for 24–26 h at 35–37 °C. The use of novobiocin is optional.

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 tip. Cover sample wells with adhesive film strips.

b. For **boot swabs**, transfer 1.0 mL of **Wash Solution** to each of 2 additional sample wells (2 well/sample) using a repeat pipette and 10 mL pipette tips. Cover all sample wells with adhesive film strips.

c. Transfer 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.

d. Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.

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. **Return samples to 35 °C incubator.**

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 Wash Solution.

g. For all samples**,** 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.

h. For **boot swabs**, transfer PickPen tips to corresponding sample wells containing Wash Solution with tips submerged, retract PickPen magnets to release particles into wash solution. Discard PickPen tips and load a new set of tips onto the PickPen device. Extend the PickPen magnets and insert tips into the strip of wells containing the Wash Solution and particles. Stir gently for 30 s while continually moving tips 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 droplets of Wash Solution. Transfer PickPen tips to the second set of sample wells containing fresh Wash Solution and gently swirl for 10 s (do not release particles into solution). Tap the PickPen tips

against the side of the sample wells to remove excess droplets of Wash Solution. Transfer particles to 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. Repeat for all boot swab samples using new tips for each strip of samples. Cover resuspension plate with adhesive film strips and continue with TEST PROCEDURE.

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 **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 Rotor-Gene in sequential order, beginning with position #1.

e. 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 each sample will be identified as **Positive** or **Negative** for *Salmonella* spp. and **Positive** or **Negative** for *S.* Heidelberg*, S.* Enteritidis and/or *S.* Typhimurium or **No Amp.** The individual serovar results (Heidelberg, Enteritidis and Typhimurium) are also presented.

Salmonella Results:

Positive: Samples are presumptive positive for *Salmonella*.

Negative: Samples are negative for *Salmonella*.

No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact Technical Service.

No.	Name	Salmonella	Salmonella Result	Assay	Kit Lot
1	Sample 1	Positive	+	Salmonella HET	abc123
2	Sample 2	Positive	+	Salmonella HET	abc123
3	Sample 3	Positive	+	Salmonella HET	abc123
4	Sample 4	Positive	+	Salmonella HET	abc123
5	Sample 5	Negative	-	Salmonella HET	abc123
6	Sample 6	No Amp	-	Salmonella HET	abc123

Salmonella HET Results:

Positive: Samples are presumptive positive for *Salmonella* Heidelberg, *S.* Enteritidis and/or *S.* Typhimurium.

Negative: Samples are negative for *Salmonella* Heidelberg, *S.* Enteritidis and/or *S.* Typhimurium.

No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact Technical Service.

No.	Name	Salmonella HET	Salmonella Result	Enteritidis Result	Typhimurium Result	Heidelberg Result	Assay	Kit Lot
1	Sample 1	Positive	+	+	-	-	Salmonella HET	abc123
2	Sample 2	Positive	+	-	+	-	Salmonella HET	abc123
3	Sample 3	Positive	+	-	-	+	Salmonella HET	abc123
4	Sample 4	Negative	+	-	-	-	Salmonella HET	abc123
5	Sample 5	Negative	-	-	-	-	Salmonella HET	abc123
6	Sample 6	No Amp	-	-	-	-	Salmonella HET	abc123

Confirmation

Samples should be incubated for 24 h prior to transfer to a secondary enrichment broth for confirmation.

Presumptive positive samples should be confirmed from the retained Assurance GDS enrichment media via: USDA – FSIS. 2014. *Microbiology Laboratory Guidebook*, 4.08. http://www.fsis.usda.gov/wps/wcm/connect/fsis-content/internet/main/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook

Storage

Store Assurance GDS for *Salmonella* HET kit components at 2–8 °C. Kit expiration is provided on the product box label.

Precautions

If possible, maintain separate work zones and dedicated equipment and supplies for sample preparation and amplification and detection.

It is recommended to utilize both positive and negative control samples.

This product is not intended for human or veterinary use. Assurance GDS for *Salmonella* HET 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 *Salmonella* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Appendix A – Enrichment Media Recipes

Tetrathionate Broth with novobiocin (TT + n)

Suspend 46 g of dehydrated Tetrathionate broth in 1 L of deionized water. Heat TT to boiling. DO NOT AUTOCLAVE. Cool to below 45 °C. Add 1 mL 1% brilliant green dye solution to TT Broth. On day of use, add 20 mL iodine-iodide solution and 8.75 mL novobiocin solution to 1 L of prepared TT broth. DO NOT REHEAT OR AUTOCLAVE MEDIUM.

Buffered Peptone Water (BPW)

Suspend 20 g of dehydrated Buffered Peptone Water (BPW) in 1 L of deionized water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

1% Brilliant Green Dye Solution

Dissolve 1 g of Brilliant Green Dye in 100 mL of sterile deionized water. Store away from light at room temperature.

Iodine-Iodide Solution

Dissolve 6 g iodine and 5 g potassium iodide in 20 mL of sterile deionized water. Store away from light at 2–8 °C.

0.45% Novobiocin Solution

Dissolve 0.45 g of Novobiocin (sodium salt) in 100 mL of sterile purified water.

Brain Heart Infusion

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

Manufacturing Entity

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