AMA Salmonella Real – Time Qualitative PCR Detection Kit

This product is intended for research use only (RUO)

Made in
SAUDI ARABIA
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Introduction and Principles of the Test

Salmonella is generally classified as follows:

- **Salmonella enterica**
  - Salmonella enterica arizonae
  - Salmonella enterica diarizonae
  - Salmonella enterica enterica
  - Salmonella enterica houtenae
  - Salmonella enterica indica
  - Salmonella enterica salamae
- **Salmonella bongori**

Each of these subspecies is further divided into multiple serovars. Our duplex, probe-based real-time PCR assay is intended for detection of all known serotypes of Salmonella in just over 1 hour (with extracted DNA as the starting material). The assay targets a 130bp-long segment of the bacterial spaQ gene, a highly conserved gene that is present only in the Salmonella species.

Target	spaQ gene	Internal Control (IC)
Detection Channel	(Salmonella)	HEX

Due to the variety of possible input samples (human, poultry, cell culture, etc.) a synthetic DNA spike-in is included in the kit to serve as the extraction internal control. This approach ensures that the extraction and amplification steps are accounted for, thus eliminating the possibility of false negatives due to erroneous extraction and/or amplification.

Kit Contents and Storage

The kit comes in a package of four tubes, as indicated in the following table. All components must be stored at -20°C. The qPCR Master Mix and AMA Oligo Mix should not be exposed to light for extended periods of time

Component	Volume (uL)
qPCR Master Mix	1,000
AMA Oligo Mix	200
Negative Control	50
Positive Control	50
Extraction Internal Control (IC)	1,000

Sensitivity

As of December 31st, 2022, there were 1,207 full sequences for all Salmonella enterica and bongori species. The oligos in our assay have perfect complementarity to 99.23% of these sequences.

Specificity

Wet-lab testing of verified bacterial cultures shows that our assay has no false positives in any of the organisms listed in the following table.

Pathogen Name (strain)			
E. coli (strain N/A)	Listeria innocua (strain N/A)	Listeria monocytogenes (strain N/A)	Enterococcus faecalis (strain N/A)

Furthermore, in-silico analysis shows that our assay has no cross-reactivity with any of the organisms listed in the table below.

Organism Name (NCBI TaxID)			
Gallus gallus domesticus (taxid:9031)	Cronobacter (taxid:413496)	Campylobacter (taxid:194)	Bacillus (taxid:1386)
Clostridium (taxid:1485)	Bacillus/Clostridium group (taxid:1239)	Staphylococcus aureus (taxid:1280)	Escherichia coli (taxid:562)
Escherichia coli O157:H7 (taxid:83334)	Legionella (taxid:445)	Yersinia (taxid:629)	Yersinia enterocolitica (taxid:630)
Listeria monocytogenes (taxid:1639)	Listeria (taxid:1637)	Legionella pneumophila (taxid:446)	Shigella (taxid:620)
Shigella flexneri (taxid:623)			

Precision

A positive and a negative samples of salmonella were run at least 5 different times using our assay. The samples were called correctly 100% of the times.

Linear dynamic range (LDR) and limit of detection (LoD)

We made a synthetic DNA construct containing the targeted salmonella sequence. From that, we constructed a standard curve to assess the linear dynamic range of our assay (from 10^8 to 10^1 salmonella copies equivalent). R^2 of the standard curve was 0.999, with PCR efficiency of 101.9%, indicating robust assay performance across a wide range of concentrations.
Moreover, we empirically tested the limit of detection of the assay, in which the assay was able to call the sample as a positive 5/5 times, to be 8 copies/reaction.

Protocol for non-bacterial culture samples

In this protocol, the Extraction Internal Control will be added to the sample during extraction, serving as a control for both the extraction and amplification steps.

1. Extract DNA or total nucleic acids from each sample as in your laboratory's approved internal protocol. However, during bacterial DNA extraction, mix 10 µL of the Extraction Internal control (IC) provided in the kit with each sample **AFTER** adding the lysis buffer. The extracted nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C immediately after extraction.

2. Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. **Do NOT exceed 3 freeze-thaw cycles!**

3. Prepare the reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):
   a. **10 µL of qPCR Master Mix / reaction**
   b. **2 µL of the AMA Oligo Mix / reaction**

*Example

Component	Per 1 reaction	X10 reactions (+5% overage)
qPCR Master Mix	10 µL	105 µL
AMA Oligo Mix	2 µL	21 µL

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

4. Transfer 12 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

6. Seal the plate with optical cap strips or an optical adhesive seal.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	Salmonella	IC
Detection Channel	FAM	HEX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

Step1: 2 minutes - 95°C (initial denaturation)

Step2: 10 seconds - 95°C (denaturation)

Step3: 30 seconds - 60°C (annealing/extension) → DATA COLLECTION

Step4: go to step2 (X39 times)

10. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

Protocol for bacterial culture samples

Bacterial cultures contain considerable amounts of DNA. Since high amounts of DNA are known to inhibit PCR, samples must be diluted before undergoing the test.
In this protocol, the Extraction Internal Control will be added to the sample after extraction, serving as a control for the amplification step, but not for the extraction step. Alternatively, extraction will be assessed either spectrophotometrically by Nanodrop or fluorescently by Qubit.

1. **Extract DNA or total nucleic acids from each sample as in your laboratory's approved internal protocol. No need to add the Extraction Internal control (IC) provided in the kit. The extracted nucleic acid should be used immediately AFTER extraction (within 30 minutes) or stored at -20°C immediately after extraction.**

2. **Measure the concentration of your DNA with Nanodrop, Qubit, or equivalent. Adjust the sample concentration to ~5-10ng/µL. Dilute your sample 1,000–10,000 fold.**

3. **Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. Do NOT exceed 3 freeze-thaw cycles!**

4. **Prepare the reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):**
   a. **10 µL of qPCR Master Mix / reaction**
   b. **2 µL of the AMA Oligo Mix / reaction**
   c. **1µL of the IC/ reaction**

*Example

Component	Per 1 reaction	X10 reactions (+5% overage)
qPCR Master Mix	10 µL	105 µL
AMA Oligo Mix	2 µL	21 µL
IC	1 µL	10.5 µL

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

5. Transfer 13 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

6. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

7. Seal the plate with optical cap strips or an optical adhesive seal.

8. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	Salmonella	IC
Detection Channel	FAM	HEX

9. On the software, define your Positive and Negative Controls.

10. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

- **Step1:** 2 minutes - 95°C (initial denaturation)
- **Step2:** 10 seconds - 95°C (denaturation)
- **Step3:** 30 seconds - 60°C (annealing/extension) → DATA COLLECTION
- **Step4:** go to step2 (X39 times)

11. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

Sample calling

For the Salmonella-specific target, the acceptable cycle threshold of detection is Ct <40, which means that Ct ≥40 should be considered as non-detected. If the Salmonella-specific target was undetected, call the sample negative only if the Extraction Internal Control Ct is below 35. If it is greater than 35, there may have been an issue with the extraction, and we strongly recommend re-extracting the sample

The following table summarizes our sample calling recommendations. A positive (+) sign indicates a Ct < 40 whereas a negative (–) sign indicates no amplification.

	SALMONELLA (FAM) CT	IC (HEX) CT	INTERPRETATION
CASE 1	+	Any	Positive infection
CASE 2	–	≤35	No infection
CASE 3	–	>35	Extraction failed; redo extraction

Run validity

For the run to be valid, the Negative Control must return a negative infection result, whereas the Positive Control must return a positive infection result (see sample calling above). If not, the whole run is considered invalid.

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Support

Support

For questions, suggestions, or technical support, feel free to contact us by email on: support@havensci.com

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Quality control

Quality control

Each lot is tested against predetermined parameters to ensure consistent performance among all lots.

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Product Manual (PD785)
Version 2.1