Simultaneous Multiplex Real Time PCR (SIMUL-qPCR)

Salmonella Assay

Assay Kit Insert for AOAC PTM Workflow

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 042001

INTENDED USE AND APPLICATION

Food processors and associated laboratories can use the SIMUL-qPCR System as a quick and reliable method for detecting *Salmonella* in environmental samples and food. All SIMUL-qPCR System assays are designed to have the same instrument run time allowing simultaneous identification of all SIMUL-qPCR System assays. In addition, each assay utilizes the power of multiplexing several targets during the same run. The SIMUL-qPCR *Salmonella* Assay incorporates a multiplex approach to identifying *Salmonella*.

The Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Salmonella* Assay has been validated by the AOAC™ Research Institute under the Performance Test Methods™ program for a variety of foods and environmental surfaces. USDA FSIS and FDA BAM methods were used for method comparison testing. The SIMUL-qPCR *Salmonella* Assay was found to be equivalent to the reference methods. The limit of detection for the SIMUL-qPCR assay is 10,000 CFU/mL of enriched sample.

PRODUCT INFORMATION

SKU	DESCRIPTION	UOM / QUANTITY
SMRT-SAL-096	SIMUL-QPCR *Salmonella* Assay Kit	1 KIT | 96 Tests/Kit

KIT COMPONENTS

SKU	DESCRIPTION	QUANTITY
KC-SMRT-LYB-25	Lysis Buffer	96 reactions | 2 bottles | 1 resealable pouch
KC-SMRT-SAL-8S	PCR Assay Tubes	96 tests | 12 strips of 8 tubes/caps | 1 resealable pouch

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PRINCIPLE

This protocol is a multifaceted approach to the detection of *Salmonella* species in a variety of food products and environmental samples. Specifically formulated media are utilized for enriching samples followed by cultural (detection plate) and rapid (qPCR) detection procedures. Buffered Peptone Water (BPW) contains necessary nutritional components for the growth of *Salmonella*. Enterohemorrhagic *E. coli* Recovery and Enrichment Broth (EREB) combine those nutritional components with additional ingredients that are necessary to selectively improve recovery and growth of *Salmonella*. The selective agents present in EREB have been optimized to efficiently inhibit competing normal bacterial flora without affecting the growth of *Salmonella* species. BPW and EREB are formulated for buffering capacity to ensure growth in a variety of matrices.

For qPCR amplification and detection, forward and reverse primers hybridize to a unique sequence *Salmonella* genomic DNA. A fluorogenic probe consisting of a DNA probe labeled with a 5`-dye and a 3`-quencher is included in the same reaction mixture. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on the real-time PCR instrument. Two unique primer and probe mixtures specific for *Salmonella* are present in this assay. The targets are found in the FAM™ and CAL Fluor® Orange 560 dye channels. An Internal Amplification Control (IAC) is included which is found in the CAL Fluor® Red 610 dye channel.

The combination of the multiple detection approaches ensures cost-effectiveness and efficiency.

ADDITIONAL MATERIALS REQUIRED

Other necessary materials not provided include:

ALL SAMPLES

- AFD *E. coli* Recovery and Enrichment Broth (EREB)
- Buffered Peptone Water (BPW)
- MyGo Pro real-time PCR instrument and installed MyGo Pro software v3.4
- Autoclave
- Distilled / deionized water
- Sterile stomacher / blender bags or equivalent with and without filter
- Stomacher / blender or equivalent
- Incubator: at 35 ± 1°C
- Incubator: at 42 ± 1°C (EREB Method)
- Incubator: at 45 ± 1°C
- Heating blocks with inserts
- Vortexer
- Calibrated thermometers
- Mini-centrifuge (optional)
- Capping / de-capping tools (optional)
- Adjustable mechanical pipettes
- Multi-channel pipette
- 1.5 ml microcentrifuge tubes or equivalent PCR-grade plastics for cell lysis
- Microcentrifuge tube racks
- Powder-free gloves
- Routine laboratory equipment

ENVIRONMENTAL SAMPLES

- AFD Quick Release Sampler Hydrated with Neutralizing and Recovery Buffer (SKU#: SC-SQRS-NRB-100)
- AFD Quick Release Sampler Hydrated with Neutralizing Buffer (SKU#: SC-SQRS-NB-100)
- AFD Quick Release Sampler Hydrated with Letheen Broth (SKU#: SC-SQRS-LB-100)
- AFD Dry Sponge with 25 mL Neutralizing Buffered Peptone Water (SKU#: SC-S-DRY-25NBPW-100)
- AFD MEMP Swab Kit (SKU#: MEMP-SWB-032)
- Other commercially available swabs

PRECAUTIONS

This product is for In Vitro diagnostic use only. Do not ingest, inhale, or allow to come into contact with skin. Observe approved biohazard precautions and aseptic techniques. Biosafety level 2 procedures should be exercised (BMBL, http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf or current site). Extreme care should be taken in handling test samples and enrichment broths. All enrichment broths, plates and other items may contain various pathogens whether or not they contain *Salmonella* species. This kit is to be used only by adequately trained and qualified laboratory personnel in a laboratory setting. All laboratory specimens should be considered infectious and handled accordingly.

PROCEDURE

Media Preparation - Autoclave Method

1. Use a clean bottle for each liter of medium preparation.
2. Shake container of dry enrichment medium before each use.
3. Measure 20.0 g of BPW powder or 37.8 g of EREB powder into the bottle and add 1 L of distilled water.
4. Constantly stir and heat solution until powder is dissolved. The acceptable pH is 7.2 ± 0.2.
5. Sterilize the bottle(s) of prepared medium by autoclaving at 121°C for 15 min.
6. Cool bottle(s) to room temperature. Media is stable at room temperature, or it can be stored at 2–8°C for up to 45 days. Keep away from light.

Media Preparation - Non-Autoclave Method

1. Prepare a sterile and clean bottle for each liter of medium preparation.
2. Shake container of dry enrichment medium before each use.
3. Measure 20.0 g of BPW powder or 37.8 g of EREB powder into the bottle and add to 1 L of sterile distilled or deionized water.
4. Constantly stir and heat solution until powder is dissolved. The acceptable pH is 7.2 ± 0.2.
5. Cool the prepared medium to the appropriate temperature (45 ± 1°C) and use immediately.

Media Preparation – Enrichment Ready Media

1. Prepare a sample bag with pre-warmed (45 ± 1°C) laboratory grade ASTM D1193 Type 2 water with the volume respective of the pouch size.
2. Tap pouch to compact media away from the tear mark. Pinch and open the perforated tear mark and empty the contents into the sample bag. Refer to Enrichment of Samples tables for acceptable media volumes.
3. Shake, stomach or hand mix until powder is dissolved.
4. Use within three to four hours while maintaining pre-warmed conditions (45 ± 1°C).

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ENVIRONMENTAL SURFACE SAMPLE PREPARATION

Sponge

1. When ready to test, pre-warm the prepared BPW to 45 ± 1°C.
2. Add the pre-warmed BPW to each sponge sample in its sample bag. Refer to Enrichment of Samples tables for acceptable media volumes.
3. Homogenize the sample for 30 seconds in a stomacher / blender or equivalent. Hand mixing is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed bag for approximately one minute.
4. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

Swab

1. When ready to test, pre-warm the prepared BPW to 45 ± 1°C.
2. Add 9 mL of the pre-warmed BPW to each swab sample in its vial.
3. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

MEMP Assay Swab

1. Refer to Molecular Environmental Monitoring Program (MEMP) Assay for *Salmonella* Detection Kit Insert.
2. Sample and process the lysate as directed in the AFD MEMP Assay.
3. Proceed to AFD MEMP assay.
4. Any samples resulting positive on the AFD MEMP Assay must be enriched and run on the SIMUL-qPCR *Salmonella* Assay for confirmation. Add the retained swab and sample solution to 9 mL of BPW and incubate for 16-20 hours at 35 ± 1°C.

FOOD SAMPLE PREPARATION

1. Aseptically sample the product or pour the rinse into a sterile bag.
2. When ready to test, pre-warm the prepared medium (45 ± 1°C).
3. Add the pre-warmed BPW or EREB to the sample. Refer to Enrichment of Samples tables for acceptable media volumes.
4. Homogenize the sample for 30 seconds in a stomacher / blender or equivalent. Hand mixing is an acceptable alternative for stomaching. To hand mix, massage each sample that is in the sealed bag for approximately one minute.
5. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

BPW ENRICHMENT OF SAMPLES

Enrichment Incubation: 35 ± 1°C for 16-20 hours

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Environmental Swab*	/Swab	9 mL ± 1 mL
AFD MEMP Reserve Swab*	/Swab	9 mL ± 1 mL
Environmental Sponge*	/Sponge	90 mL ± 10 mL
Poultry Carcass or Parts Rinse	/30 mL	30 mL ± 5 mL
Raw Ground Poultry	/375 g	1 L ± 50 mL
RTE Cooked Poultry	/375 g	1 L ± 50 mL
Dry Pet Food**	/375 g	2 L ± 100 mL
Pasteurized Liquid Eggs	/100 g	900 mL ± 18 mL
Peanut Butter	/25 g	225 mL ± 15 mL
Frankfurter/Sausage	/25 g	225 mL ± 15 mL

*Validated surfaces are stainless steel, plastic, rubber, ceramic tile, and sealed concrete

**BPW for dry pet food enrichment should be pre-warmed to 35 ± 1°C, not 45 ± 1°C

EREB ENRICHMENT OF SINGLE SAMPLES USING AFD SIMUL-qPCR *SALMONELLA* PROTOCOL

Enrichment Incubation: 42 ± 1°C for 10-18 hours

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Raw Ground Beef	/375 g	1 L ± 50 mL
Raw Beef Excision Sample N60	/375 g	1 L ± 50 mL

* AOAC PTM Method is not validated for 10 hours.

ASSAY PREPARATION

1. At the end of the enrichment phase, proceed to the cell lysis protocol for single samples.
2. The qPCR set up and data entry should be completed prior to transferring samples.
3. Prepare equipment:
   - Turn on the heating blocks to 95 ± 3ºC as measured by a calibrated thermometer.
   - Power on the qPCR instrument and create run file from SIMUL-qPCR template. The SIMUL-qPCR template contains the required cycle.
4. After removing the aliquot required for lysis, return the enrichment(s) to the incubator.

SET UP THE qPCR INSTRUMENT

1. Refer to the PCR Set Up Guide and the MyGo Pro PCR Software Manual for detailed instructions.
2. Select the AFD Template files to begin run configuration. The AFD template file contains all of the PCR machine settings required to perform the run. Do not change any settings under the “Experiment”, “Run Profile”, or “Data” tabs.
3. Under the “Samples” tab, populate the sample fields according to the well placement / position.
4. Include the kit lot number in the “Notes” field.
5. Add the targets to the sample(s).
6. After cell lysis and loading, Click “Start Run”.

CELL LYSIS PROTOCOL OF ENRICHED SAMPLES

After incubation, follow the steps below for cell lysis:

1. Label one 1.5 mL microcentrifuge tube or equivalent PCR-grade plastic tube per sample.
2. Aseptically pipette 400 µL of lysis buffer into each tube. Return lysis buffer to storage (2-8ºC).
3. From the enrichment broth, pipette 5 µL into the prepared tube. Cap the tube(s) and vortex.
4. Heat the closed tubes for 10 minutes at 95 ± 3ºC in the heat block.
5. Remove the closed tubes from the heat block.
6. Allow tubes to cool for 5 minutes at room temperature.
7. Proceed directly to SIMUL-qPCR *Salmonella* Assay or hold lysate in a refrigerator (2-8ºC) up to 48 hours before proceeding to SIMUL-qPCR *Salmonella* Assay.

SET UP THE SIMUL-qPCR ASSAY

1. Arrange strips of PCR tubes according to your run file.
2. Using caution, remove the caps from the strip of tubes.
3. Pipette 20 µL of lysate into the sample wells of the PCR test strip, ensuring the pellet is hydrated. PCR pellets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.
4. Place the caps onto each tube and press down to seal each lid.
5. Make sure each lid is tightly secured before running on the PCR machine.
6. If air bubbles are present, carefully flick reaction tubes until no air bubbles remain.
7. Briefly spin down the reaction tubes in a mini-centrifuge.
8. Load the qPCR instrument and start the run.

REVIEW RESULTS OF SIMUL-qPCR ASSAY

Once the SIMUL-qPCR Assay run is complete, data is analyzed automatically by the software. The software analyzes any DNA amplification data and will display a Cq value for any sample that amplifies. Amplification in the FAM™ channel or the CAL Fluor® Orange 560 channel indicates *Salmonella*. Only a Cq value that has a typical sigmoidal curve or the beginning of the curve is considered positive for the target. When a Cq value is not obtained, the result is negative for the target provided a Cq value is present in the CAL Fluor® Red 610 channel for the IAC.

PRODUCT STORAGE AND EXPIRATION

Store the sealed kit at 2 - 8°C. Once opened, protect kit components from moisture and light by keeping container(s) tightly closed after each use. Re-seal qPCR tubes in re-sealable foil pouch. The expiry date is indicated on the package.

DISPOSAL

Dispose of all materials used and the enrichment medium by autoclaving or according to approved practices. Ensure that all biohazard waste is disposed of according to local, municipal, provincial, state and/or federal regulations.

TECHNICAL INFORMATION

If you have any questions or experience issues with this kit, please contact our support staff via email (support@appliedfooddiagnostics.com). For more information about Applied Food Diagnostics, Inc., please visit us at our website (**www.appliedfooddiagnostics.com**).

QUALITY CONTROL

All products manufactured by Applied Food Diagnostics, Inc. are incorporated into a quality assurance program from the time the raw materials arrive in the factory through to marketing the end product. Each batch of end product undergoes quality control, and is only marketed if it complies with acceptance criteria. Documentation concerning production and verification of each batch is archived. A Certificate of Analysis of this quality control and Safety Data Sheets are available on the web at **www.appliedfooddiagnostics.com**.

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