



**MOLECULAR ENVIRONMENTAL MONITORING PROGRAM (MEMP) ASSAY FOR SALMONELLA DETECTION KIT INSERT FOR AOAC PTM WORKFLOW**



**INTENDED USE AND APPLICATION:**

Food processors and associated laboratories can use the MEMP assay as a quick and reliable method for detecting *Salmonella* on stainless steel, plastic, rubber, ceramic tile, and sealed concrete environmental samples. All MEMP assays are designed to have the same instrument run time allowing simultaneous identification of all MEMP assay targets. The MEMP assay incorporates a real-time PCR approach to identifying *Salmonella* cells expressed from a swab sample.

The Molecular Environmental Monitoring Program (MEMP) Assay for *Salmonella* Detection has been validated by the AOAC™ Research Institute under the Performance Test Methods™ program for stainless steel, plastic, rubber, ceramic tile, and sealed concrete environmental samples. The FDA BAM method were used for method comparison testing. The MEMP Assay was found to be equivalent to the reference methods. The limit of detection for this MEMP assay is 10 CFU/swab.

**PRODUCT INFORMATION**

SKU	DESCRIPTION	UOM / QUANTITY
MEMP-SAL-032	MEMP Assay for <i>Salmonella</i> Detection	1 Kit   32 Tests

**KIT COMPONENTS**

SKU	DESCRIPTION	QUANTITY
KC-MEMP-SAL-LT	<i>Salmonella</i> Lysis Tubes	32 Reactions   32 Lysis Tubes   1 Resealable Pouch
KC-MEMP-RB-10	Resuspension Buffer	32 Reactions   1 Bottle   1 Resealable Pouch
KC-MEMP-RS-8S	Resuspension Strips	32 Tests   4 Strips of 8 Tubes & Caps   1 Resealable Pouch
KC-MEMP-SAL-8S	PCR Assay Tubes	32 Tests   4 Strips of 8 Tubes & Caps   1 Resealable Pouch



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**PRINCIPLE**

During PCR amplification, forward and reverse primers hybridize to a unique sequence of *Salmonella* genomic DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5'-dye and a 3'-quencher. 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. Unique and specific primer and probe mixtures are present in this assay. The *Salmonella* target is found in the FAMTM dye channel. An Internal Amplification Control (IAC) is included and is found in the CAL Fluor® Red 610 dye channel.

**ADDITIONAL MATERIALS REQUIRED**

Other necessary materials not provided include:

**ENVIRONMENTAL SWAB SAMPLES**

- AFD MEMP Swab Kit (SKU#: MEMP-SWB-032) Included 32 of the Following:
  - Sterile Flocked Collection Swab (SKU#: SC-SFCS-SWAB)
  - Neutralizing and Recovery Buffer Bottles (SKU#: KC-MEMP-NRB-001)
  - Swab Collection Tube with Swab Expression Solution (SKU#: KC-MEMP-SES-001)
- MyGo Pro Real-Time PCR Instrument and Installed MyGo Pro Software v3.4
- Heating Blocks
- Calibrated Thermometers
- Vortexer (Optional)
- Mini-Centrifuge (Optional)
- Capping / De-Capping Tools (Optional)
- Adjustable Mechanical Pipettes
- Multi-Channel Pipette
- Powder-Free Gloves

**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 1 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 assay 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**

**Environmental Surface Sample Preparation**

1. Use the AFD Sterile Flocked Collection Swab and dip into one AFD Neutralizing and Recovery Buffer Bottle to hydrate the swab.
2. Swab the surface to be tested with one side of the swab in a horizontal direction (approximately 1 inch) and with the other side in a vertical direction (approximately 1 inch) back and forth (one stroke back and one stroke forward) to swab the entire area.
3. Open the Swab Collection Tube with Swab Expression Solution and insert the flocked swab tip. Spin or twist the swab several times to express any potential cells into the expression solution.
4. At the 30mm break-point of the flocked swab, break off the swab tip into the expression solution and close the tube.
5. If not proceeding to the assay within 2 to 3 hours, the swab can be placed under refrigeration for up to 30 hours.

**ASSAY PREPARATION**

1. After expressing the cells in the expression solution, proceed to the cell lysis protocol.
2. The qPCR set up and data entry should be completed prior to transferring samples.
3. Prepare equipment:
  - Turn on one heating block to 37 ± 2°C as measured by a calibrated thermometer.
  - Turn on second heating block to 95 ± 3°C 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.

**SET UP THE qPCR INSTRUMENT**

Refer to the PCR Set Up Guide for detailed instructions on setting up the qPCR instrument and related instructions.

**CELL LYSIS PROTOCOL**

1. Label one lysis tube with lysis pellet per sample.
2. Aseptically pipette 500 µL of the expression solution into the labeled lysis tube and close the tube. Reserve / retain the balance of expression solution, containing the expressed cells and swab tip for confirmation.
3. Vortex, shake or mix the sample to hydrolyze the pellet.
4. Place the lysis tube(s) on the heat block and incubate at 37 ± 2°C for 15 minutes.
5. Transfer the lysis tube(s) to the second heat block and incubate at 95 ± 3°C for 10 minutes.
6. Remove the lysis tube(s) from the heat block and allow to cool for 5 minutes at room temperature.
7. Proceed directly to the next step or hold lysate under refrigerated temperatures (2-8°C) up to 48 hours before proceeding to MEMP *Salmonella* assay.
8. Arrange resuspension tube(s) and aseptically open the lids.
9. Pipette 180 µL of the resuspension solution into the resuspension tube(s).
10. Pipette 20 µL of the lysed solution into the resuspension tube(s).

**SET UP THE MEMP 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 resuspension 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 AND INTERPRET THE RESULTS OF MEMP ASSAY

Once the MEMP 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 indicates *Salmonella* species. 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.

All positive results are potential positives and require confirmation. Potential positives should be enriched and analyzed according to AFD SIMUL-qPCR *Salmonella* species and monocytogenes Assay. Any potential positives from the AFD SIMUL-qPCR *Salmonella* species and monocytogenes Assay should be culturally confirmed using the FDA BAM (<https://www.fda.gov/food/laboratory-methods-food/bam-detection-and-enumeration-listeria-monocytogenes>).

Note: Some positive results may be difficult to culturally confirm due to low levels of target cells, high levels of background flora, or a combination of these factors. Contact Technical Support for additional information.

### 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](mailto:support@appliedfooddiagnostics.com)). For more information about Applied Food Diagnostics, Inc. please visit us at our website ([www.appliedfooddiagnostics.com](http://www.appliedfooddiagnostics.com)).

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