Simultaneous Multiplex Real Time PCR (SIMUL-qPCR)

Top 7 STEC (*E. coli* O157:H7, O26, O45, O103, O111, O121, O145)

Assay Collection Insert for AOAC PTM Workflow

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 022001

INTENDED USE AND APPLICATION

Food processors and associated laboratories can use the SIMUL-qPCR System as a quick and reliable method for detecting the Top 7 Shiga toxin-producing *E. coli* in raw beef trim, raw ground beef, and spunbonded polyolefin sampling sheets. 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 Top 7 STEC Assay incorporates a multiplex approach to identifying *E. coli* O157:H7, O26, O45, O103, O111, O121, O145, Shiga toxin and intimin genes.

The Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) Top 7 STEC Assay has been validated by the AOAC™ Research Institute under the Performance Test Methods™ program for raw beef trim, raw ground beef, and sampling sheets. The USDA FSIS MLG method was used for method comparison testing. The SIMUL-qPCR Top 7 STEC Assay was found to be equivalent to the reference method. The limit of detection for this SIMUL-qPCR assay is 10,000 CFU/mL of enriched sample.

PRODUCT INFORMATION

SKU	DESCRIPTION	UOM / QUANTITY
SMRT-T7-096	SIMUL-qPCR Top7 STEC Assay Collection	1 KIT | 96 Tests/Kit

KIT COMPONENTS

SKU	DESCRIPTION	DETECTS
SMRT-ECH7-096	SIMUL-qPCR *E. coli* O157:H7 Kit	*E. coli* O157:H7
SMRT-STXEAE-096	SIMUL-qPCR Shiga Toxin (stx) and Intimin (eae) Gene STEC Kit	Shiga toxin gene Intimin gene
SMRT-O26O103-096	SIMUL-qPCR *E. coli* O26 and *E. coli* O103 STEC Kit	O26, O103
SMRT-O111O145-096	SIMUL-qPCR *E. coli* O111 and *E. coli* O145 STEC Kit	O111, O145
SMRT-O45O121-096	SIMUL-qPCR *E. coli* O45 and *E. coli* O121 STEC Kit	O45, O121

APPLIED FOOD DIAGNOSTICS
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PRINCIPLE

Enterohemorrhagic *E. coli* Recovery and Enrichment Broth (EREB) is a selective medium, specifically optimized for single-step recovery and enrichment of Enterohemorrhagic *E. coli* (EHEC) from raw beef trim, ground beef, and sampling sheets. The medium can also be used for the recovery and enrichment of *Salmonella* as a single-enrichment method alongside EHEC in beef samples.

During PCR amplification, forward and reverse primers hybridize to unique sequences of the target's 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. The collection is composed of 5 unique PCR reaction tubes with multiple STEC targets which are to be run simultaneously but may be processed individually. The table below specifies the targets and corresponding fluorescence channels.

KIT NAME	FAM™	CAL Fluor® Orange 560	CAL Fluor® Red 610
SIMUL-qPCR *E. coli* O157:H7 Kit	*E. coli* O157:H7	No Target	IAC
SIMUL-qPCR Shiga Toxin (stx) and Intimin (eae) Gene STEC Kit	Intimin (eae) gene	Shiga toxin (stx) gene	IAC
SIMUL-qPCR *E. coli* O26 and *E. coli* O103 STEC Kit	*E. coli* O103	*E. coli* O26	IAC
SIMUL-qPCR *E. coli* O111 and *E. coli* O145 STEC Kit	*E. coli* O145	*E. coli* O111	IAC
SIMUL-qPCR *E. coli* O45 and *E. coli* O121 STEC Kit	*E. coli* O45	*E. coli* O121	IAC

ADDITIONAL MATERIALS REQUIRED

Other necessary materials not provided include:

ALL SAMPLES

- AFD EHEC Recovery and Enrichment Broth (EREB)
- MyGo Pro real-time PCR instrument and installed MyGo Pro software v3.4
- Autoclave
- Distilled / deionized water
- Sterile sampling bag
- Incubator: at 42 ± 1°C
- Incubator: at 45 ± 1°C
- Mini-centrifuge (optional)
- Heating Blocks with inserts
- Vortexer
- Calibrated thermometer
- 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
- Positive and Negative Controls

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 may contain various pathogens whether or not they contain Shiga toxin-producing *E. coli*. The collection 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 37.8 g of 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 37.8 g of 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. 1 Prepare a sample bag with one liter of pre-warmed (45 ± 1°C) laboratory grade ASTM D1193 Type 2 water.
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 or hand mix until powder is dissolved.
4. Use within three to four hours while maintaining pre-warmed conditions (45 ± 1°C).

FOOD SAMPLE PREPARATION

1. Aseptically sample the product and place in a sterile bag.
2. When ready to test, pre-warm the prepared EREB to 45 ± 1°C.
3. Add the pre-warmed EREB to the sample. Refer to Enrichment of Samples tables for acceptable media volumes.
4. Hand mix by massaging each sample that is in the sealed bag for approximately one minute to homogenize each sample.
5. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

SAMPLING SHEET PREPARATION

1. When ready to test, pre-warm the prepared EREB to 45 ± 1°C.
2. Add the pre-warmed EREB to the sample. Refer to Enrichment of Samples tables for acceptable media volumes.
3. Hand mix by massaging each sample that is in the sealed bag for Approximately one minute to homogenize each sample.
4. Incubate the sample. Refer to Enrichment of Samples tables for Enrichment conditions.

EREB ENRICHMENT OF SINGLE SAMPLES USING AFD SIMUL-qPCR TOP 7 STEC 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
Spunbonded Polyolefin Sampling Sheet	/sheet	200 mL ± 15 mL

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”, “Data” tab.
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 from a culture broth:

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 labeled tube. Return lysis buffer to storage (2-8ºC).
3. Pipette 5 µL of the enrichment broth 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 assay or hold lysate in a refrigerator (2-8ºC) up to 48 hours before proceeding to SIMUL-qPCR assay.

Note: Adhering to proper pipetting guidelines for qPCR micro-volumes is critical (i.e. Pipette with smooth and deliberate action; hold the pipette vertically at all times. Immerse the pipette tip only slightly to avoid coating the outside of the tip with excess liquid that may be inadvertently transferred during dispensing. Pipette the initial volume directly to the bottom of the receiving container while lifting the pipette upward slowly so as not to introduce bubbles to the dispensed solution. Add additional volumes to the initial volume using the same technique.)

SET UP THE SIMUL-qPCR ASSAY

1. Select the PCR tubes of the assay(s) for the desired testing being performed. Assays can be run individually or concurrently.
2. Arrange strips of PCR tubes according to your run file.
3. Using caution, remove the caps from the strip of tubes.
4. 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.
5. Place the caps onto each tube and press down to seal each lid.
6. Make sure each lid is tightly secured before running on the PCR machine.
7. If air bubbles are present, carefully flick reaction tubes until no air bubbles remain.
8. Briefly spin down the reaction tubes in a mini-centrifuge.
9. Load the qPCR instrument and start the run.

REVIEW AND INTERPRET THE RESULTS OF SIMUL-qPCR ASSAY

Once the run is complete, results are analyzed automatically by the software. The software analyzes any DNA amplification data and will display a Cq value for any sample that amplifies. 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 confirmation is recommended. Enriched samples can be confirmed using either the FSIS MLG method or the FDA BAM method using the enrichment broth, stored at 2-8°C, according to your laboratory SOP for your sample type.

Note: Some positive results may be difficult to culturally confirm due to low levels of *E. coli* 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. Reseal qPCR tubes in resealable 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**).

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