



RapidChek® CONFIRM™ STEC IMS

Confirmation Protocol for Potential Positive Samples

RapidChek® CONFIRM™ Non-O157 STEC IMS Confirmation Kit, 10001433

Test Kit Includes:

- O26 Specific Immunomagnetic Bead Solution, 5 mL
- O45 Specific Immunomagnetic Bead Solution, 5 mL
- O103 Specific Immunomagnetic Bead Solution, 5 mL
- O111 Specific Immunomagnetic Bead Solution, 5 mL
- O121 Specific Immunomagnetic Bead Solution, 5 mL
- O145 Specific Immunomagnetic Bead Solution, 5 mL
- 3 Packs PBS-T
- Package Insert

Kit for the detection and confirmation of O26, O45, O103, O111, O121 and O145 *E. coli* from meat samples. Confirmation materials for 100 presumptive positives per serogroup.

RapidChek® CONFIRM™ O157 STEC IMS Confirmation Kit, 10001731

Test Kit Includes:

- O157 Specific Immunomagnetic Bead Solution, 5 mL

Also Required:

- Magnetic Rack, 20 Position, 10001402
- Sample Tubes, 2 mL, 10003687

Also Available:

- RapidChek® *E. coli* O157 (including H7)
- RapidChek® SELECT™ *Salmonella*
- RapidChek® SELECT™ *Salmonella* Enteritidis
- RapidChek® CONFIRM™ *Salmonella* Enteritidis IMS
- RapidChek® *Listeria*
- RapidChek® *Listeria* NextDay™





1

Prepare IMS reagent

Re-suspend the working stock of IMS beads by repeated inversion of the vial.



2

Transfer secondary enrichment

Transfer 1 mL aliquots of the potential positive enrichment to 2 mL sample tubes.



Storage of reagents

Store all reagents at 2 – 8 °C. Do not freeze.

PBS-T wash buffer preparation

Dissolve the contents of the Phosphate-Buffered Saline, 20% Tween 20 (PBS-T) Packet in 1 L of deionized water. Filter sterilize using a 0.2 µm filter. Store at 2 – 8 °C for up to 3 months.

3

Transfer IMS reagent

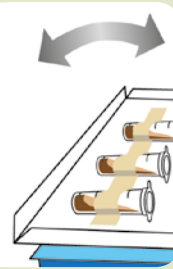
Transfer 0.05 mL of each IMS Reagent into samples tubes containing the sample enrichment. Vortex briefly to mix.



4

Incubate the sample

Incubate the samples at room temperature with rocking for 15 minutes.



5

Concentrate the sample

Place the sample tubes onto the magnetic separation rack for 5 minutes.



6

Remove unbound material

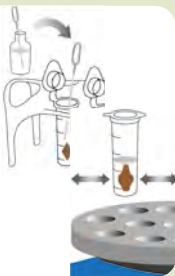
Using a pipette, remove the liquid from the sample tube being careful not to touch the IMS beads on the side of the tube closest to the magnetic source.



7

First wash

Remove the sample tubes from the magnetic rack. Add 1 mL of PBS-T (Wash Buffer) to the sample tubes and vortex briefly to mix and re-suspend the IMS beads.



8

Second separation

Place the sample tube back on the magnetic rack for 5 minutes.



9

Remove unbound material

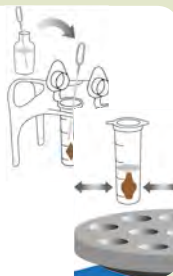
Using a pipette, remove the liquid from the sample tube being careful not to touch the IMS beads on the side of the tube closest to the magnetic source. Repeat steps 7 – 9 for a total of 3 washes.



10

Reconstitute washed sample

After the final wash step, reconstitute the sample with 1 mL of Wash Buffer and vortex briefly to mix.



11

Plate sample

Plate 0.1 mL to selective agar plates (i.e. Modified Rainbow Agar). In addition, it is recommended that an aliquot of the original bead solutions be diluted 1:10 in wash buffer prior to plating 0.1 mL in order to aid in single colony isolation. Alternatively, quadrant streak 0.01 mL to selective agar plates. Proceed to the confirmation protocol as described in "Detection and Isolation of non-O157 Shiga-toxin producing *Escherichia coli* (STEC) from Meat Products" which can be found at: <http://www.fsis.usda.gov/PDF/MLG-5B.pdf>

