Increase ease and confidence

Simultaneous detection of *E. coli* O157:H7 and Big 6 STEC
The best option for rapid detection

For beef producers, rapid and accurate results when screening products for STEC (Shiga toxigenic *E. coli*) and other pathogens can bring substantial economic benefits. You can release product sooner, avoid unnecessary product diversions and production shutdowns, as well as minimize risk to your brand and reputation.

Current reference methods for detecting STEC in beef samples are accurate and sensitive but can be slow and labor-intensive. Alternative rapid methods can show reduced accuracy and often require large volumes of proprietary media, making them a risky and uneconomic option.

The Applied Biosystems™ RapidFinder™ STEC Detection Workflow enables screening for *E. coli* O157:H7 and six strains of Shiga toxigenic *E. coli*—the Big 6 STECs—in as few as 12 hours, providing a rapid, validated workflow for STEC detection in raw ground beef and raw beef trim.

The RapidFinder STEC Detection Workflow is a two-stage real-time PCR method, designed to accurately detect and confirm presence of *E. coli* O157:H7 and any of the Big 6 serogroups of non-O157 STEC defined by the USDA Food Safety and Inspection Service (FSIS) as adulterants in the American beef industry (serogroups O26, O45, O103, O111, O121 & O145).

The RapidFinder STEC Detection Workflow provides:

- **Greater confidence in results**—optimized workflow, co-developed with USDA ARS, showed equivalent performance to the USDA-MLG reference method in AOAC-RI validation studies
- **Simplified workflow**—simultaneous detection of *E. coli* O157:H7 and any of the Big 6 non-O157:H7 *E. coli* in 375g raw ground beef and raw beef trim samples with a system to fit within your existing testing program
- **Rapid product release**—same-day detection of pathogenic STEC and clearance of STEC-negative product in as few as 12 hours with presumptive positives confirmed with just one additional PCR test (~1 hour)
- **Minimized costs, space requirements and handling steps**—single enrichment step in non-proprietary enrichment broth

A simple, accurate detection solution

The RapidFinder STEC Detection System comprises two multiplexed assays (Figure 1):

![Assay Format](image)

**A. RapidFinder STEC Screening Assay**

- **3 targets:** stx1/2, eae, O157 indicator (plus IPC & ROX™)
- **Observed Signal(s):**
  - Positive for a Big 6
  - Positive for *E. coli* O157:H7
  - Potential O157:H7

**B. RapidFinder STEC Confirmation Assay (only used when the RapidFinder STEC Screening Assay indicates a positive result)**

- **2 targets:** Big 6, O157:H7 (plus IPC & ROX™)

Figure 1. Two-stage assay design clears negative samples for multiple STEC in a single reaction. (A) The initial assay screens for Shiga toxin 1 (stx1/2—all 4 subtypes) and Shiga toxin 2 (stx2—all 7 subtypes), Intimin adherence protein (eae—all variants), and potential O157:H7 presence. (B) If samples are positive for stx and eae or for *E. coli* O157:H7, then the confirmation assay confirms presence/absence of Big 6 or *E. coli* O157:H7. Both screening and confirmation assays use the same DNA sample preparation. All reactions include internal positive control (IPC) and ROX passive reference.

* O157a - *E. coli* O157:H7 Screening Assay target
** O157b - *E. coli* O157:H7 Confirmation Assay target
A rapid, reliable alternative for food producers

Two AOAC Research Institute (AOAC-RI) Performance Tested Methods\textsuperscript{SM} (PTM) certified workflow options:

**Workflow 1**: Uses automated sample preparation on the Applied Biosystems™ MagMax™ Express-96 Magnetic Particle Processor and Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit. Up to 96 samples can be processed together, from enrichment to RapidFinder STEC Screening Assay results, in approximately 12 hours. One additional hour is required for the RapidFinder STEC Confirmation Assay results. Suitable for laboratories running a high test throughput.

**Workflow 2**: Uses the Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit - Extra Clean with Proteinase K for manual PCR sample preparation with spin columns, providing screening release in approximately 18 hours. Ideal for lower test throughput.

**Simpler data, better data**

The RapidFinder STEC Detection Workflow provides test result data in a simple format that is designed to reduce the likelihood of errors, minimize frustration, and free up resources. With minimal hands-on time, automated capabilities, and “smart” software, the Applied Biosystems™ RapidFinder™ Express v. 2.0 Software simplifies user interaction (Figure 2).

When integrated with the RapidFinder STEC Detection Workflow, and run on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument, RapidFinder Express v2.0 automates data interpretation and generates user instructions on how to proceed with a sample:

- **RapidFinder STEC Screening Assay result**:  
  - Negative—clear sample  
  - Requires confirmation—proceed with RapidFinder STEC Confirmation Assay

- **RapidFinder STEC Confirmation Assay result**:  
  - Positive—sample contains a pathogenic STEC

The software interface shows simple presence/absence calls in RapidFinder Express v.2.0 or, for more advanced users, displays raw data files using the 7500 Fast System SDS v.1.4.2.1 Software.

*Figure 2. Versatile software for automated data interpretation. The RapidFinder Express software version 2.0 displays results by location in both (A) Table View and (B) Layout View. (C) Screening Assays can be connected with the Confirmation Assays using the Import Screen results feature.*
Additional products for STEC detection, confirmation and identification

For rapid O-group identification from the same DNA sample preparation used with RapidFinder STEC Screening and Confirmation Assays, use Applied Biosystems™ TaqMan® MLG STEC Assay Beads. Each assay is multiplexed to detect and identify two of the Big 6 non-O157 STEC and follow the USDA FSIS MLG published designs.

For screening, identification and confirmation of non-O157 STEC according to USDA FSIS MLG 5B we can also supply many of the high quality reagents that have been optimized for this method and also used with many other “gold standard” protocols.

**Applied Biosystems™ TaqMan® Environmental Master Mix v2.0**
- Proven performance in food enrichment samples which contain qPCR inhibitors
- Validated qPCR master mix listed in the USDA MLG 5B.01 (Nov 2011) protocol for detection of non-O157 STEC in beef samples
- High sensitivity with both single target and multi-target qPCR assays

**Applied Biosystems™ Dynabeads™ for Immunomagnetic Separation (IMS) of pathogenic E. coli**
- Dynabeads are the original and most trusted name in IMS technology and can make your detection method simpler and more consistent
- Dynabeads for E. coli serogroups O26, O103, O111, O45, O121 and O145 are the validated IMS reagents listed in the USDA MLG 5B.01 (Nov 2011) protocol for detection of non-O157 STEC in beef samples
- Dynabeads E. coli O157:H7 reagent is listed as part of an optimal method in numerous regulatory confirmation methods around the world such as FDA-BAM, USDA MLG, ISO, Health Canada and Japanese Ministry of Health

**7500 Fast PCR Instrument**
- Trusted performance for consistent amplification of up to five target dye multiplex assays
- Validated qPCR instrument listed in the USDA MLG 5B.01 (Nov 2011) protocol for detection of non-O157 STEC in meat samples
- Single PCR instrument platform for both your rapid STEC screening and USDA MLG confirmation testing when used in conjunction with the RapidFinder STEC Detection Workflow

**Dedicated service and support**

Our extensive network of service and support teams, comprised of highly-experienced scientists, is designed to offer rapid response and best-in-class technical support. Included with the purchase of a 7500 Fast System are full instrument installation and performance verification by a certified field service engineer, training by one of our field application specialists and a one-year warranty.

We also offer extended and flexible service options designed with your laboratory in mind—from preventative maintenance packages to compliance services (i.e. IQ/OQ, OQ/IPV).
### Ordering information

<table>
<thead>
<tr>
<th>Description</th>
<th>Pack size</th>
<th>Order code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RapidFinder STEC Detection Workflow Enrichment media</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trytone Soya Broth (dehydrated culture medium)</td>
<td>500g</td>
<td>CM0129B</td>
</tr>
<tr>
<td></td>
<td>2.5Kg</td>
<td>CM0129R</td>
</tr>
<tr>
<td></td>
<td>5Kg</td>
<td>CM1029T</td>
</tr>
<tr>
<td>Trytone Soya Broth (prepared culture medium)–US only</td>
<td>4x1 litre</td>
<td>R112740</td>
</tr>
<tr>
<td></td>
<td>4x1 litre</td>
<td>R112737</td>
</tr>
<tr>
<td><strong>RapidFinder STEC Detection Workflow Nucleic Acid Extraction Kits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PrepSEQ Rapid Spin Sample Preparation Kit–Extra Clean with Proteinase K</td>
<td>100 reactions</td>
<td>4426715</td>
</tr>
<tr>
<td>PrepSEQ Nucleic Acid Extraction Kit*</td>
<td>100 reactions</td>
<td>4480466</td>
</tr>
<tr>
<td>PrepSEQ Nucleic Acid Extraction Kit*</td>
<td>300 reactions</td>
<td>4428176</td>
</tr>
<tr>
<td>MagMax Express 96 Deep Well Processor</td>
<td></td>
<td>4400079</td>
</tr>
<tr>
<td><strong>RapidFinder STEC Detection Workflow Assays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RapidFinder STEC Screening Assay</td>
<td>96 reactions</td>
<td>4476868</td>
</tr>
<tr>
<td>RapidFinder STEC Confirmation Assay</td>
<td>96 reactions</td>
<td>4476901</td>
</tr>
<tr>
<td>RapidFinder STEC Screening Assay Starter Kit–with PrepSEQ Nucleic Acid Extraction Kit*</td>
<td>96/100 reactions</td>
<td>4480467</td>
</tr>
<tr>
<td><strong>7500 Fast 96-well Real-Time PCR Instrument, Computers, Software &amp; Service packages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7500 Fast Real-Time PCR System Package, Laptop**</td>
<td></td>
<td>A30299</td>
</tr>
<tr>
<td>7500 Fast Real-Time PCR System Package, Desktop**</td>
<td></td>
<td>A30304</td>
</tr>
<tr>
<td>RapidFinder Express v2.0 and SDS 1.4.2 Software for 7500 Fast System</td>
<td></td>
<td>A28811</td>
</tr>
<tr>
<td>IQ/OQ Service for 7500 Fast System</td>
<td></td>
<td>4365572</td>
</tr>
<tr>
<td>OQ/IPV Service for 7500 Fast System</td>
<td></td>
<td>4365571</td>
</tr>
<tr>
<td>Complete Service Plan for 7500 Fast System</td>
<td></td>
<td>7500AMTCOM</td>
</tr>
<tr>
<td>Assurance Service Plan for 7500 Fast System</td>
<td></td>
<td>7500AMTASS</td>
</tr>
<tr>
<td><strong>TaqMan Real-Time PCR Assays for confirmation of specific non-O157 STEC O-groups (MLG design)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan STEC O103 &amp; O145 PCR Assay Beads, MLG</td>
<td>96 reactions</td>
<td>4485063</td>
</tr>
<tr>
<td>TaqMan STEC O26 &amp; O111 PCR Assay Beads, MLG</td>
<td>96 reactions</td>
<td>4485064</td>
</tr>
<tr>
<td>TaqMan STEC O45 &amp; O121 PCR Assay Beads, MLG</td>
<td>96 reactions</td>
<td>4485065</td>
</tr>
<tr>
<td><strong>Additional items suitable for use when following USDA FSIS MLG 5B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trytone Soya Broth (modified) with Novobiocin</td>
<td>2.5Kg</td>
<td>R458634</td>
</tr>
<tr>
<td></td>
<td>10Kg</td>
<td>R458636</td>
</tr>
<tr>
<td>TaqMan Environmental Master Mix 2.0</td>
<td>200 reactions</td>
<td>4396838</td>
</tr>
<tr>
<td>Dynabeads anti-E. coli O157 Magnetic Beads†</td>
<td>1ml</td>
<td>71003</td>
</tr>
<tr>
<td>Dynabeads anti-E. coli O157 Magnetic Beads†</td>
<td>5ml</td>
<td>71004</td>
</tr>
<tr>
<td>Dynabeads EPEC/VTEC O26 Magnetic Beads</td>
<td>2ml</td>
<td>71013</td>
</tr>
<tr>
<td>Dynabeads MAX EPEC/VTEC O45 Kit</td>
<td>100 reactions</td>
<td>A14631</td>
</tr>
<tr>
<td>Dynabeads MAX EPEC/VTEC O45 Kit</td>
<td>200 reactions</td>
<td>A14683</td>
</tr>
<tr>
<td>Dynabeads EPEC/VTEC O103 Magnetic Beads</td>
<td>2ml</td>
<td>71011</td>
</tr>
<tr>
<td>Dynabeads EPEC/VTEC O111 Magnetic Beads</td>
<td>2ml</td>
<td>71009</td>
</tr>
<tr>
<td>Dynabeads MAX EPEC/VTEC O121 Magnetic Beads</td>
<td>100 reactions</td>
<td>A14632</td>
</tr>
<tr>
<td>Dynabeads MAX EPEC/VTEC O121 Magnetic Beads</td>
<td>200 reactions</td>
<td>A14684</td>
</tr>
<tr>
<td>Dynabeads EPEC/VTEC O145 Magnetic Beads</td>
<td>2ml</td>
<td>71007</td>
</tr>
</tbody>
</table>

* Used with the MagMax Express 96 Deep Well Processor for high-throughput automated workflow

** Includes: 7500 Fast instrument, Computer, RapidFinder Express v2.0 and SDS 1.4.2 Software, Capping tool, Precision plate holder, Calibration kits and Block balance tubes

† Dynabeads E. coli O157:H7 reagent is listed as an optimal method in numerous regulatory confirmation methods around the world such as FDA-BAM, USDA MLS, ISO, Health Canada and Japanese Ministry of Health
Access a more complete solution for each step of your microbiological food testing workflow

For more information on rapid, accurate STEC detection for your laboratory, please contact one of our support specialists or go to thermofisher.com/stec

Contact Information:
microbiology@thermofisher.com  993-145
USA +1 800 255 6730  LT2193A
International +44 (0) 1256 841144  April 2016