MicroSEQ® *E. coli* O157:H7 Detection Kit

Fast, flexible, and highly specific

- Fast time-to-results—as little as 8 hours
- Specificity based on genome sequencing
- 1–3 CFU sensitivity
- Flexible workflows
- Easy-to-use lyophilized format

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A Public Health Threat

Escherichia coli is a common bacterium found in the intestines of healthy animals. There are hundreds of strains of *E. coli.* Most of them are harmless, but a few are pathogenic. *E. coli* O157:H7 is one of the most virulent.

E. coli O157:H7 is responsible for tens of thousands of cases of foodborne illness each year, including chronic diarrhea and hemorrhagic colitis, and may lead to hemolytic-uremic syndrome (HUS). HUS can lead to the destruction of red blood cells and kidney failure, which can cause stroke, seizures, and death. Infants, the elderly, and immunocompromised individuals are the most susceptible to HUS.

In the United States, outbreaks of *E. coli* O157:H7 infection have been traced to contaminated ground beef, spinach, juice, and cookie dough. Such an outbreak not only is a threat to human health but also can have devastating consequences on food producers in terms of cost, reputation, and loss of brand equity.

The Testing Challenge

Detection of *E. coli* O157:H7 in the food supply is a public health priority worldwide. As a result, food producers and government agencies have taken an active role in testing for *E. coli* O157:H7 in raw materials, production processes, and finished products. The challenge is to specifically detect *E. coli* O157:H7, but not the nonvirulent serotypes, at very low levels and with fast time-to-results, so that immediate and appropriate action can be taken if contamination is found.

The MicroSEQ® *E. coli* O157:H7 Detection Kit meets the challenge, with a fast, specific, and sensitive real-time PCR assay that is easy to use and easy to integrate into laboratory workflows.

The Cross-Reactivity Problem

The nearest phylogenetic neighbor of *E. coli* O157:H7 is *E. coli* O55:H7. *E. coli* O55:H7 can coexist with *E. coli* O157:H7 but is rarely associated with disease. Because their genomes are so closely related, the presence of *E. coli* O55:H7 can produce a false positive result when running some of the assays currently available for detection of *E. coli* O157:H7.

Enhanced Specificity

To design an assay that is highly specific for *E. coli* O157:H7, the genome of *E. coli* O55:H7 was sequenced using the next-generation Applied Biosystems® SOLiD™ platform and compared to the publicly available sequence of *E. coli* O157:H7. Sequences specific to *E. coli* O157:H7 were then used as targets to design the MicroSEQ® *E. coli* O157:H7 Detection Kit.

The MicroSEQ® *E. coli* O157:H7 Detection Kit gains additional specificity through the use of TaqMan® chemistry. Unlike SYBR® Green chemistry, which has only target-specific primers, TaqMan® chemistry has both primers and probes that are specific to the target, an additional way to help prevent false positives.

Time-to-Results Is Critical

Bacteria can grow and spread quickly. If undetected, even low levels of *E. coli* O157:H7 contamination can lead to an outbreak that poses a serious threat to human health. It can also become a nightmare for food producers, who may be forced to recall and destroy contaminated products, shut down operations, and face lost revenue, damaged reputations, fines, and litigation.

Rather than risk an outbreak, food producers often test for *E. coli* O157:H7 and hold products in inventory until the results are confirmed. While this makes sense from a prevention and containment perspective, it can create bottlenecks that hamper productivity and increase costs. With the MicroSEQ® *E. coli* O157:H7 Detection Kit these delays are unnecessary.

Eliminate Testing Bottlenecks

The MicroSEQ® *E. coli* O157:H7 Detection Kit is fast. The assay takes as little as 8 hours for a 25 g sample of beef or produce and only 18 hours for a 375 g ground beef or beef trim sample. By comparison, culture-based assays, long considered the gold standard for pathogen detection, take up to 5 days. Immunoassays take up to 2 days. Molecular tests that lack specificity for *E. coli* O157:H7 are too ambiguous to support timely containment.

Figure 1. No mixing required. Lyophilized reagents reduce hands-on time and help ensure reproducible results.

High Sensitivity Is Essential

To ensure food safety, the ability to detect extremely low levels of *E. coli* O157:H7 is essential. This is difficult to achieve with methods such as cell culture and immunoassay.

Along with specificity and fast time-to-results, sensitivity is one of the major advantages of the MicroSEQ® *E. coli* O157:H7 Detection Kit. Sensitivity of the kit is 1–3 colony forming units (CFU) per 25 g of sample. The assay has the same level of sensitivity for up to 375 g of ground beef or beef trim. The limit of detection of the kit is 10^3 CFU/mL of sample, after enrichment.

Lyophilized and Optimized

For maximum ease of use and reduced chances of operator error, the reagents in the MicroSEQ® *E. coli* O157:H7 Detection Kit are lyophilized into ready-to-use, pre-formatted assay beads. The beads hold the active enzyme, the target-specific primer and probe set, an Internal Positive Control (IPC), and other reagents for PCR. No electrophoresis or post-PCR processing is required. All the operator has to do is to prepare the assay beads, add the samples and controls, and run the test.

Added Productivity

Increased demand for testing adds burdens of time and cost on food-testing laboratories. The MicroSEQ® *E. coli* O157:H7 Detection Kit is designed to save time and money while also improving laboratory efficiency.

- **Operator training is minimal:** From sample preparation to interpretation of results, the entire procedure has been reduced to a few simple steps that can be learned quickly.
- **Use the same equipment as other MicroSEQ® kits:** In addition to the MicroSEQ® *E. coli* O157:H7 Detection Kit, MicroSEQ® kits are also available for *Listeria* spp., *Listeria monocytogenes*, and *Salmonella* spp.

Demonstrated Performance

The MicroSEQ® *E. coli* O157:H7 Detection Kit assays are based on fast real-time PCR. The assay specifically detects low levels of *E. coli* O157:H7 in a range of sample types, including:

- Ground beef
- Beef trim
- Produce
- Fruit juices

- **Test for multiple pathogens at the same time:** All MicroSEQ® assays can be run together on the same 96-well plate regardless of enrichment conditions. In addition, when running the MicroSEQ® *E. coli* O157:H7 assay using BPW enrichment, samples from the same enrichment can also be tested with the MicroSEQ® *Salmonella* spp. assay.

Optimized Sample Preparation

The MicroSEQ® *E. coli* O157:H7 Detection Kit is optimized for use with the PrepSEQ™ Sample Preparation Kits. The PrepSEQ™ kits remove PCR inhibitors, enhance assay performance, and enable the user to enrich and prepare high-quality samples from a broad range of food sample types.

- For automated high-throughput applications, the PrepSEQ™ Nucleic Acid Extraction Kit prepares high-quality microbial DNA and RNA from broth cultures when used with the Applied Biosystems® MagMAX™ Express-96 Sample Preparation System.
- For lower sample numbers, the PrepSEQ™ Rapid Spin Sample Preparation Kit provides a simple, cost-effective way to prepare DNA from broth cultures.

Fast, Actionable Answers

RapidFinder™ Express Software simplifies the assay procedure by guiding the user through every step. At the end of the assay, the software presents an easy-to-read screen with the results of each reaction. Results are clearly displayed and can be labeled with flags, notifications, and prompts that enable the operator to quickly interpret the data and take appropriate action.

A Complete Solution

The Applied Biosystems MicroSEQ® *E. coli* O157:H7 Detection Kit is part of the complete food-testing solution offered by Life Technologies. The kit includes everything required to run 96 reactions. All components have been designed for rapid implementation in food-testing laboratories and performance-verified to make detection as fast, easy, and reliable as possible. Everything is provided ready to use.

- **Sensitive and specific:** The assay is designed to provide maximum sensitivity on the 7500 Fast Real-Time PCR System. It has been designed to be highly specific for *E. coli* O157:H7 detection.
- **Ready to use:** The active enzyme, reagents, primers and probes, and internal positive control are lyophilized into preformatted assay beads. No mixing is required.
- **Streamlined sample preparation:** A choice of sample preparation kits helps ensure high-quality assay results.
- **Software-guided:** Application-specific RapidFinder™ Express Software guides the user through each step of the procedure—from run-file setup to final results.
- **AOAC-Certified:** The full workflow including the MicroSEQ® *E. coli* O157:H7 spp. Detection Kit, PrepSEQ™ Sample Preparation Kits, 7500 Fast instrument, and RapidFinder™ Express Software has earned the Performance Tested Methods℠ certification from the AOAC Research Institute.

Workflow Flexibility

Different laboratories have different requirements. To better meet the needs of food-testing laboratories, the MicroSEQ® *E. coli* O157:H7 Detection Kit offers the flexibility of three different workflows. All three can be performed by nontechnical personnel with minimal training or prior experience and can be quickly integrated into your lab.

1. **Fast Time-to-Results**
   - 8–10 hr time-to-results
   - 25 g sample
   - BHI enrichment
2. **Standard Media**
   - 18 hr time-to-results
   - 25 g sample
   - BPW enrichment
3. **Larger Volumes**
   - 18 hr time-to-results
   - 375 g sample of ground beef or beef trim
   - BPW enrichment

Figure 2. The MicroSEQ® *E. coli* O157:H7 Detection Kit offers the flexibility of three easy-to-use workflows.

Figure 3. Software-Guided Procedure. After sample enrichment and preparation, RapidFinder™ Express Software guides the user through the entire assay procedure with on-screen instructions. Amplification, detection, data collection, and analysis are fully automated.

Resources for Food Safety

As the world leader in real-time PCR, Life Technologies is committed to providing the food industry with improved tools for pathogen detection. The MicroSEQ® *E. coli* O157:H7 Detection Kit is part of a growing family of fast and convenient food pathogen detection tools that utilize lyophilized reagents, application-specific software, optimized sample preparation, and fast real-time PCR instrumentation. We also provide responsive, knowledgeable applications consulting, support, training, and technical service.

For more information about the MicroSEQ® *E. coli* O157:H7 Detection Kit and our other solutions for food pathogen testing, please contact your local Life Technologies sales representative or visit us at www.appliedbiosystems.com/foodsafety.

ORDERING INFORMATION

Description	Part Number
MicroSEQ® Pathogen Detection Kits	
MicroSEQ® *E. coli* O157:H7 Detection Kit	4427409
MicroSEQ® *E. coli* O157:H7 Detection kit with Protocol and QRC	4445654
MicroSEQ® *E. coli* O157:H7 Detection Starter Kit with PrepSEQ™ Rapid Spin Kit Sample Preparation Kit	4445657
MicroSEQ® *E. coli* O157:H7 Detection Starter Kit with PrepSEQ™ Nucleic Acid Extraction Kit	4445656
MicroSEQ® *Listeria monocytogenes* Detection Kit	4403874
MicroSEQ® *Listeria* spp. Detection Kit	4427410
MicroSEQ® *Salmonella* spp. Detection Kit	4403930
Sample Preparation	
PrepSEQ™ Nucleic Acid Extraction Kit	4428176
PrepSEQ™ Rapid Spin Sample Preparation Kit	4407760
PrepSEQ™ Rapid Spin Sample Preparation Kit with Proteinase K	4426714
PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean	4413269
PrepSEQ™ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K	4426715
Instrumentation and Software	
Food Pathogen Detection System Package: Applied Biosystems® 7500 Fast Real-Time PCR System with PC Tower and RapidFinder™ Express Software	4445785
Food Pathogen Detection System Package: Applied Biosystems® 7500 Fast Real-Time PCR System with Notebook Computer and RapidFinder™ Express Software	4445787

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