

9815 ANSR for *E. coli* Enrichment Broth

Intended Use

ANSR for *E. coli* Enrichment Broth is used in the **Neogen ANSR for *E. coli* O157:H7** test method for the presumptive identification of ***E. coli* O157:H7** from raw ground beef, raw beef trim, spinach and sprout irrigation water.

Product Summary and Explanation

E. coli O157:H7 continues to be an important public health problem. *Escherichia coli* O157:H7 is an enterohemorrhagic serotype of the bacterium *Escherichia coli* and can cause illness, typically through the consumption of contaminated food. While most clinical cases can result in diarrhea and abdominal cramps with little to no fever, infection with this type of pathogenic bacterium may lead to hemorrhagic diarrhea and potentially, to kidney failure, primarily in children under five years of age and the elderly. The illness usually resolves in five to ten days. It can also be asymptomatic.

In an AOAC Research Institute Performance Tested Method Study, ANSR for *E. coli* O157:H7 was found to be an effective procedure for detection of this potential pathogen. Samples producing positive ANSR results may be confirmed by streaking the enrichment cultures onto *E. coli* O157:H7 selective/differential agar media and continuing with identification of presumptive colonies following standard methods.^{1,2}

Principles of the Procedure

The ANSR for *E. coli* O157:H7 test is an amplified nucleic acid assay. The method is based on nicking enzyme amplification reaction (NEAR) technology. See the package insert for P/N 9822 for a complete outline of the process. The sample is enriched in 9815 ANSR for *E. coli* Enrichment Broth at an elevated temperature of $42 \pm 1^\circ\text{C}$ for a sample-specific time period, then a small aliquot (50ul) of the enriched culture is added to lysis tubes, lysis reagent is added, the tubes are incubated in a heat block and the lysis reaction proceeds. After the lysis reaction time (up to one hour), 50ul of the lysed sample is added to the ANSR reaction tubes, vortex-mixed to distribute the lysed sample onto the reaction reagents, then inserted into the ANSR reader. After 10 minutes, the ANSR software will indicate a positive or negative results.

Precautions

1. For laboratory use.
2. **IRRITANT.** Irritating to eyes, respiratory system, and skin.

Directions

Preparation directions are dependent upon the type of sample tested. Refer to the ANSR for *E. coli* O157:H7 test system package insert for complete instructions.

ANSR for *E. coli* Enrichment Broth

Rehydrate 39.6 g of ANSR for *E. coli* Enrichment Broth with 1 L sterile water pre-warmed to 42°C . **Use the rehydrated medium the same day as prepared. Do not autoclave.**

SAMPLE ENRICHMENT

For sprout irrigation water

1. Measure out 125ml of sprout rinse water in a Stomacher-type bag.
2. Add 1.125 L **ANSR for *E. coli* Enrichment Broth** (Neogen item 9815) (pre-warmed to 42°C) to the bag. Swirl to mix.
3. Incubate the culture at $42 + 1^\circ\text{C}$ for **12–24 hours**.

For leafy green vegetables

1. Weigh out 200 g of sample in a Stomacher-type bag.
2. Add 600 ml of **ANSR for *E. coli* Enrichment Broth** (pre-warmed to 42°C) to the bag. Swirl to mix.
3. Incubate the culture at 42 + 1°C for **24–26 hours**.

For raw ground beef

1. Weigh out 325 g of sample in a Stomacher-type bag.
2. Add 1.625L of **ANSR for *E. coli* Enrichment Broth*** (pre-warmed to 42°C) to the bag. Use manual or automated stomacher method to disperse clumps.
3. Incubate the culture at 42 + 1°C for **12–24 hours**.
*See package insert; some samples may require the use of 7167 TSB, modified with Acid Digest of Casein; see package insert for 9822 for complete details.

For raw beef trim

1. Weigh out 325 g of sample in a Stomacher-type bag.
2. Add 1.825L of **ANSR for *E. coli* Enrichment Broth*** (pre-warmed to 42°C) to the bag. Use manual or automated stomacher method to disperse clumps.
3. Incubate the culture at 42 + 1°C for **12–24 hours**.
*See package insert; some samples may require the use of 7167 TSB, modified with Acid Digest of Casein; see package insert for 9822 for complete details.

Quality Control Specifications

Dehydrated

Appearance: Medium is homogenous, free-flowing and light beige.

Rehydrated

Appearance: Light amber, brilliantly clear to clear with none to light precipitate.

Solubility: 39.6 grams dissolves in one liter of water.

pH: pH of the re-hydrated medium should be 7.0 ± 0.2.

Performance:

This medium is tested for growth support and recovery of minimal inoculum of the target organism into a raw beef slurry with results for presumptive detection read on Reveal for *E. coli* O157:H7 (9754 or 9755) devices and confirmation of the recovery on 7103 Eosin Methylene Blue Agar.

Microorganism	Reveal for <i>E. coli</i> test results	EMB
<i>E. coli</i> O157:H7 ATCC 35150	Positive	Growth; colonies have green metallic sheen
<i>E. coli</i> O157:H7 ATCC 43888	Positive	Growth; colonies have green metallic sheen
<i>E. coli</i> O157:H7 ATCC 43895	Positive	Growth; colonies have green metallic sheen
<i>E. coli</i> O157:H7 ATCC 43889	Positive	Growth; colonies have green metallic sheen
<i>E. coli</i> ATCC 25922	Negative	Growth; colonies have green metallic sheen

Test Procedure

Refer to the ANSR for *E. coli* O157:H7 package inserts for complete details.

Results

Refer to ANSR for *E. coli* O157:H7 package inserts for complete details. The presumptive identification of *E. coli* O157:H7 must be confirmed with biochemical and serological procedures.^{1,2}

Storage

Store ANSR media in a sealed container at 15 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Keep container tightly closed; protect from moisture.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color.

Limitations of the Procedure

1. Use re-hydrated medium within 6 hours of preparation.
2. Do not autoclave ANSR media or use expired media.
3. Sterile water should be brought to the indicated temperature before use when using ANSR media.
4. The presumptive identification of *E. coli* O157:H7 must be confirmed by further testing.^{1,2}

Packaging

ANSR for *E. coli* Enrichment Broth

Code No. 9815 500 g

References

1. USDA-FDA (2014). Bacteriological Analytical Manual Chapter 4A. *Escherichia coli* [http://www.fda.gov/Food/Food Science Research/Laboratory Methods/ucm070080.htm](http://www.fda.gov/Food/Food%20Science%20Research/Laboratory%20Methods/ucm070080.htm)
2. USDA-FSIS (2015) Microbiology Laboratory Guidebook, chapter 5.09

Technical Information

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