

## TRYPTIC SOY BROTH, MODIFIED with NOVIOBIOCIN & ACID DIGEST OF CASEIN (7731)

### Intended Use

Tryptic Soy Broth, Modified with Novobiocin & Acid Digest of Casein is used for the selective enrichment of enterohemorrhagic *E. coli* in foods. Conforms to USDA Formulation<sup>1</sup> with Novobiocin supplementation.

### Product Summary and Explanation

There are four recognized classes of enterovirulent *E. coli* that cause gastroenteritis in humans.<sup>2</sup> Enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7, makes up one of the four classes.<sup>2</sup> *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine.<sup>2</sup> *E. coli* O157:H7 infection often cause severe bloody diarrhea and abdominal cramps. The infection can also cause a complication called Hemolytic Uremic Syndrome (HUS). The toxins destroy red blood cells and can lead to kidney failure. About 2 – 7% of infections lead to HUS, particularly in children below the age of 5 and the elderly.<sup>3</sup>

The first major outbreak of *E. coli* O157:H7 was in 1982, and traced to contaminated hamburgers.<sup>2</sup> Other known sources of infection include sprouts, lettuce, salami, unpasteurized milk, juice and/or swimming in or drinking contaminated water.<sup>3</sup> Tryptic Soy Broth, Modified with Novobiocin & Acid Digest of Casein is used to enrich food samples suspected of having low levels of EHEC during pathogen testing.<sup>1,4,5</sup>

### Principles of the Procedure

Enzymatic Digest of Casein, Acid Digest of Casein, and Enzymatic Digest of Soybean Meal provide nitrogen, vitamins and minerals to stimulate growth in Tryptic Soy Broth Modified with Novobiocin & Acid Digest of Casein. Sodium Chloride maintains the osmotic balance of the medium. Dipotassium Phosphate is the buffering agent. Dextrose is the carbon energy source. Bile Salts No. 3 and Novobiocin are used as selective agents to inhibit bacterial growth.

### Formula / Liter

Enzymatic Digest of Casein .....	17.0 g
Acid Digest of Casein.....	10.0 g
Sodium Chloride .....	5.0 g
Dipotassium Phosphate .....	4.0 g
Enzymatic Digest of Soybean Meal .....	3.0 g
Dextrose.....	2.5 g
Bile Salts No. 3 .....	1.5 g
Novobiocin .....	0.020 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Precaution

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

### Directions

1. Suspend 43.02 g of the medium in 1000 mL of purified water.
2. Autoclave at 121°C for 15 minutes.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and light to medium beige.

**Prepared Appearance:** Prepared medium is brilliant to clear, none to light precipitate, and light to medium amber.

**Expected Cultural Response:** Tryptic Soy Broth Modified with Novobiocin & Acid Digest of Casein was inoculated with the test organisms listed below. These organisms were incubated at the appropriate atmosphere and temperature and examined for growth after 18 – 22 hours.

Microorganism	Approx. Inoculum (CFU)	Growth
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Growth
<i>Escherichia coli</i> ATCC® 11775	10 - 300	Growth
<i>Escherichia coli</i> O157:H7 ATCC® 35150	10 - 300	Growth
<i>Escherichia coli</i> O157:H7 ATCC® 43888	10 - 300	Growth
<i>Escherichia coli</i> O157:H7 ATCC® 43889	10 - 300	Growth
<i>Escherichia coli</i> O157:H7 ATCC® 43895	10 - 300	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Markedly suppressed to inhibited

The organisms listed are the minimum that should be used for quality control testing.

### **Test Procedure**

Refer to appropriate references for specific procedures on the recovery of pathogenic *E. coli*.

### **Results**

Refer to appropriate references for test results on the detection and enumeration of pathogenic *E. coli*.

### **Storage**

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

### **Expiration**

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### **Limitations of the Procedure**

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

### **Packaging**

#### **Tryptic Soy Broth, Modified with Novobiocin & Acid Digest of Casein**

<b>Code No.</b>	<b>7731A</b>	<b>500 g</b>
	<b>7731B</b>	<b>2 kg</b>
	<b>7731C</b>	<b>10 kg</b>

### **References**

1. **USDA.** 2008. Food Safety and Inspection Service, Detection, Isolation and Identification of *Escherichia coli* O157:H7 from meat products. MLG 5.04, USDA/FSIS Microbiology Laboratory Guidebook, Washington D.C.
2. **U.S. FDA.** Center for Food Safety & Applied Nutrition. 2001. Food pathogenic microorganisms and natural toxins handbook. *Escherichia coli* O157:H7. College Park, MD.
3. [http://www.cdc.gov/ncidod/abmd/diseaseinfo/escherichiacoli\\_g.htm](http://www.cdc.gov/ncidod/abmd/diseaseinfo/escherichiacoli_g.htm).
4. **Hill, W.E., A. R. Datta, P. Feng, K. A. Lampel, and W. L. Payne.** 1998. FDA Bacteriological analytical manual, 8<sup>th</sup> ed. Identification of Foodborne Bacterial Pathogens by Gene Probes. AOAC International, Gaithersburg, MD.
5. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm).

### **Technical Information**

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.