

Medium used for the isolation and cultivation of Enterohemorrhagic *Escherichia coli* O157:H7 in foods. *Equally use with MFDS (MB-T0789K).

• CONTENTS (Liter)

Peptone from Casein	17.0 g
Peptone from Soymeal	3.0 g
Sodium Chloride	1.5 g
Bile Salts No.3	2.5 g
D(+)-Glucose	2.5 g
Dipotassium Phosphate	4.0 g
Final pH = 7.3 \pm 0.2 at 25°C	

PROCEDURE

Suspend 30.5 G of powder in 1 L of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize by autoclave at 121°C for 15 minutes. Cool to 45 - 50°C in water bath. Aseptically add 2 vials of Novobiocin supplement (MB-N1821). Mix well. Pour into tubes.

Novobiocin supplement

1 vial contents (each vial is sufficient for 500 mL of medium) Novobiocin 0.01 g

INTERPRETATION

TSB (Tryptic Soy Broth) Modified is a medium used for the isolation and cultivation of Enterohemorrhagic *Escherichia coli* O157:H7 in foods. Peptone from casein, peptone from soymeal provide nitrogen, carbon, vitamins and minerals. Sodium chloride maintains the osmotic balance. Bile salts No.3 and novobiocin supplement inhibits the growth of Gram-positive bacteria. D(+)-Glucose is the carbohydrate. Dipotassium phosphate is the buffering agent.

TECHNIC

Inoculate the specimen using a sterile needle to the medium. Incubate at 35 \pm 2°C for 18 - 24 hours. Refer appropriate references for recommended test procedure.

• QUALITY CONTROL FOR USE

<u>Dehydrated medium</u> Appearance: free-flowing, homogeneous Color: light beige <u>Prepared medium</u> Appearance: clear Color: light amber Incubation conditions: $35 \pm 2^{\circ}$ C / 18 - 24 hours

Microorganism	ATCC	Inoculum CFU	Growth
Escherichia coli O157:H7	35150	50-100	good
Escherichia coli	25922	50-100	good
Enterococcus faecalis	29212	≥10 ³	partially inhibited
Staphylococcus aureus	25923	≥10 ³	partially inhibited

STORE

The powder is very hygroscopic. Store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label. Store prepared medium at 2 - 8°C.

REFERENCES

- 1. U.S. FDA. Center for Food Safety & Applied Nutrition. 2001. Food pathogenic microorganisms and natural toxins handbook. Escherichia coli O157:H7. College Park, MD.
- Hill, W.E., A. R. Datta, P. Feng, K. A. Lampel, and W. L. Payne. 1998. FDA Bacteriological analytical manual, 8th ed. Identification of Foodborne Bacterial Pathogens by Gene Probes. AOAC International, Gaithersburg, MD
- 3. DIN Deutsches Institut für Normung e.V., Nachweis von Escherichia coli 0157 in Lebensmitteln, DIN 10167.
- 4. FDA Bacteriological Analytical Manual, 8th Edition/1995, Chapter 4. Escherichia coli and the Coliform Bacteria, page 4.20: Isolation Methods for Enterohemorrhagic E. coli (EHEC).
- 5. S.D. Weagant, J.L. Bryant, K.G. Jinneman, An improved rapid technique for isolation of Escherichia coli 0157:H7 from foods, J. Food Prot., 58; 7-12 (1995)
- 6. Refer to the MFDS.

PACKAGE

