TECHNICAL DATA SHEET

MODIFIED TRYPTO-CASEIN SOY BROTH (MTSB)

DETECTION OF *ESCHERICHIA COLI* O157

1 INTENDED USE

Modified Tryptone-Soy Broth (mTSB) is an enrichment medium destined for the detection of pathogenic serotypes of *Escherichia coli*, in particular the O157:H7 serotype, in food products and other potentially contaminated samples from animal origin.

The typical composition of the broth corresponds to that defined in the International standard ISO 16654.

2 HISTORY

The serotype O157 of *Escherichia coli* is a pathogenic serotype, identified for the first time in 1982 as the agent responsible for hemorrhagic colitis. Infected feedstocks appear to the the principle source of human contamination. Bovine meat, pork, poultry and certain non-pasteurized milks have been incriminated in toxi-infections linked to *Escherichia coli* O157. This microorganism can also be isolated from potatoes, cider and tap water.

Many authors have successfully used modified Tryptone-Soy Broth for the selective enrichment of *Escherichia coli* O157:H7. The base formula of Tryptone-Soy Broth was originally modified by addition of 1.5 g/L of dipotassium phosphate and 1.5 g/L of bile salts. In 1987, Doyle *et al.* proposed the addition of 20 mg/L of novobiocin to reinforce selectivity and to enhance detection of *Escherichia coli* O157:H7.

3 PRINCIPLES

The nutrient base combining Tryptone, papaic digest of soybean meal and glucose provides for optimal growth of *Escherichia coli* O157:H7.

The combined presence of bile salts and novobiocin ensures the inhibition of Gram-positive microorganisms and certain Gram-negative microorganisms such as *Proteus*.

The dipotassium phosphate maintains pH and thus increases recovery capacity.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Tryptone ..... 17,0 g
- Papaic digest of soybean meal ..... 3,0 g
- Glucose ..... 2,5 g
- Bile salts n°3 ..... 1,5 g
- Sodium chloride ..... 5,0 g
- Dipotassium phosphate..... 4,0 g
- Novobiocin..... 20,0 mg

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

For 33 g of dehydrated base media BK150

- Tryptone ..... 17,0 g
- Papaic digest of soybean meal..... 3,0 g
- Glucose ..... 2,5 g
- Bile salts n°3 ..... 1,5 g
- Sodium chloride ..... 5,0 g
- Dipotassium phosphate ..... 4,0 g

For one vial of supplement BS056

- Novobiocin ..... 40 mg

For one vial of supplement BS033

- Novobiocin ..... 10 mg

5 PREPARATION

- Dissolve 33,0 g of dehydrated media (BK150) in 1 liter of distilled or demineralized water.
- Stir slowly, until complete dissolution.
- Dispense into vials, at 225 mL per vial.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.
- Reconstitute the Novobiocin 40 mg selective supplement (BS056) with 20 mL of sterile distilled water or the Novobiocin 10 mg selective supplement (BS033) with 5 mL.
- Mix or vortex in order to insure complete homogenization, while avoiding the formation of foam.
- Aseptically add 2,25 mL of reconstituted selective supplement per 225 mL vial, or 0,9 mL per vial of 90 mL.
- Homogenize thoroughly.

✓ **Reconstitution :**
33,0 g/L

✓ **Sterilization :**
15 min at 121 °C

✓ **Supplement rehydration :**
20 mL sterile water +BS056
5 mL sterile water +BS033

✓ **Add to base :**
2,25 mL / 225 mL

6 INSTRUCTIONS FOR USE

- Aseptically add X g of product to test per vial of 9X mL of broth.
- Mix well.
- Incubate at 41,5 ± 1 °C for 6 hours then again for 12 to 18 hours.

✓ **Inoculation :**
1 :10

✓ **Incubation :**
6 to 24 h at 41.5°C

7 RESULTS

- After 6 hours of incubation, aseptically remove an aliquot of the culture and perform immunomagnetic separation, followed by isolation onto CT-SMAC agar (BK150).
- Prolong the incubation 12 to 18 additional hours. Repeat the immunomagnetic separation, followed by the isolation onto CT-SMAC agar.

8 QUALITY CONTROL

Dehydrated media : cream white powder, free-flowing and homogeneous.
Novobiocin Selective Supplements : white pellet, after reconstitution gives rise to a colorless, limpid solution.
Prepared (complete) media : amber, limpid solution.

Typical culture response after 24 hours of incubation at 41,5 °C, followed by subculture onto CT-SMAC agar :

Microorganisms		Growth
Escherichia coli O157:H7	WDCM 00014	> 10 characteristic colonies
+ *Escherichia coli*	WDCM 00013	
+ *Proteus mirabilis*	WDCM 00023	
Escherichia coli O157:H7	NCTC 13126	> 10 characteristic colonies
+ *Enterobacter aerogenes*	WDCM 00175	
+ *Staphylococcus aureus*	WDCM 00034	
Staphylococcus aureus	WDCM 00034	Inhibited
Enterococcus faecalis	WDCM 00087	Inhibited

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Novobiocin Selective Supplements : 2-8 °C
The expiration dates are indicated on the label.

Rehydrated Novobiocin Supplement (*) : 30 days at 2-8 °C
Base media prepared in vials (*) : 180 days at 2-8 °C
Complete media prepared in vials (*) : 30 days at 2-8 °C.
.(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without Novobiocin) :
500 g bottle BK150HA
Novobiocin Selective Supplement :
8 x 40 mg vials BS05608
10 x 10 mg vials BS03308

11 BIBLIOGRAPHY

Doyle, M.P., and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Envir. Microbiol. 53: 2394-2396.

Feldsine, P.T., M.T. Falbo-Nelson, S.L. Brunelle, and R.L. Forgey. 1997. Assurance Enzyme Immunoassay for the Detection of Enterohemorrhagic *Escherichia coli* O157:H7 in Selected Foods: Collaborative Study. Journal of AOAC International. 80: 530-543.

NF EN ISO 16654. Juillet 2001. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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