

## Specification

Sterile selective supplement used for the isolation of *E.coli O157:H7*

## Presentation

10 Freeze dried vials  
Vial  
with: 3 ± 0.1 g

### Packaging Details

22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.

### Shelf Life

49 months

### Storage

2-25 °C

## Composition

Compositon (g/vial)

Novobiocin..... 0.010

**NOTE :** Each vial is sufficient to supplement 500ml of medium Base: TSB Tryptone Soy Broth or Semisolid Rappaport-Vassiliadis Modified.

Reconstitute the original freeze-dried vial by adding :

Sterile Distilled Water..... 6 ml

## Description /Technique

### Description:

The Novobiocin is used in different culture media like TSB Tryptone Soy Broth or Semisolid Rappaport-Vassiliadis Modified, in order to isolate *E.coli O157:H7* and/or *Salmonellas spp.* enhancing the inhibition of Gram-positive microorganism.

### Technique:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial with a sterile diluent in aseptic conditions and add it to 500ml of medium base cooled to room temperature.

Do not overheat once supplemented.

Pour the complete medium into suitable containers.

Inoculate and incubate according to normatives and specifications.

Presuntive isolation of the required microorganism must be confirmed by further tests.

For *E.coli O157* enrichment - Use Tryptone Soya broth

Following the instructions of ISO 16654:2001, the detection of *E.coli O157:H7* is developed in four consecutive steps:

-Enrichment of the sample in TSB+Novobiocin

-Separation and concentration with immunomagnetic techniques

-Isolation in two different agars: the first one CT SMAC/MacConkey Sorbitol and a second one freely chosen by the user.

-Confirmation of the presumptive colonies of *E.coli O157:H7* with biochemical tests.

Reconstitute the vial with sterile distilled water, pre-warmed to aprox. 37 °C and add to 500 ml of sterilized broth base cooled to room temperature.

Incubate the tubes tightly closed in aerobic atmosphere at 42±/2 °C for 24h.

Follow the instructions detailed in the ISO

## Quality control

### Physical/Chemical control

Color: White-Gray      pH: at 25°C

### Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Distribute the complete medium, cooled to 50 °C, into 10 ml tubes

Incubate according instructions for complete medium indicated in COMPOSITION.

Anaerobiosis. Incubation at 44 ± 1 °C during 21 ± 3h.

### Microorganism

*E. coli* 0157:H7 (non toxg.) ATCC® 700728 WDCM 00014

*Sph. aureus* ATCC® 25923, WDCM 00034

### Growth

Good

Inhibited

### Sterility Control

Add 5 ml of the sample to:

100 ml TSB and 100 ml Thioglycollate.

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

## Bibliography

ISO 16654:2001 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli* O157.

De Smedt, J. M. et al. (1991) Int. J. Food Micro. 13 301-308