

Specification

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

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 °C

Composition

Compositon (g/vial)

Novobiocin..... 0.010

Note: Each vial is sufficient to supplement 500 ml of medium Base: TSB or 1L MSRV.

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 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 instrucions 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

Note: for MSRV medium the quantity of 10 mg is for 1L of Base medium.

Quality control**Physical/Chemical control**

Color : White-Gray

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**Growth**

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

Good

Stph. aureus ATCC® 25923, WDCM 00034

Inhibited

Sterility control

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

Check at 7 days after incubation in same conditions.

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

· UNE-EN ISO Standard 6579-1:2017/A1 (2021) Microbiology of food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 : Detection of *Salmonella* spp. - Modification 1: Extension of the incubation temperature range, modification of the status of Annex D and correction of the composition of the MSRV and SC media