
SELENITE-CYSTINE BROTH

SELECTIVE ENRICHMENT FOR *SALMONELLA*

1 INTENDED USE

Selenite-Cystine broth is used for the selective enrichment of *Salmonella* in milk and dairy products but also for the characterization of residual sludge. It can also be used in animal health for the detection of *Salmonella Gallinarum* or in water testing.

The typical composition responds to that defined in the standards NF U47-101 & NF U47-102; NF EN ISO 6579-1/A1; NF EN ISO 19250; FD/CEN/TR 15215-2.

2 HISTORY

Guth, confirming the initial observations of Handel and Thodorascu, used sodium selenite as a selective agent in an enrichment broth for *Salmonella Typhi*, after demonstrating the toxicity of the substance towards *Escherichia coli*. Leifson extended the work of Guth by developing the formula of a selenite broth for the selective enrichment of *Salmonella Typhi* and *Paratyphi* from pathological samples by showing that the number of coliform bacteria decreased during the first 12 hours of incubation, while the number of typhoid bacilli rapidly increased in parallel. This broth is a modification of the original formula of Leifson. This formulation containing cystine was proposed by the Food and Drug Administration as being one specifically destined for the detection of *Salmonella* in food products.

3 PRINCIPLES

The quantity of selenite inhibits microorganisms other than salmonellae, especially coliform bacteria and enterococci. *Pseudomonas* and *Proteus* are partially inhibited.

Dipotassium phosphate maintains the pH constant and reduces the toxicity of selenium in order to increase the recovery capacity of the medium.

4 TYPICAL COMPOSITION

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

For 1010 mL of media:

- Tryptone	5.0 g
- Lactose.....	4.0 g
- Disodium hydrogenophosphate	10.0 g
- Sodium hydrogenoselenite.....	4.0 g
- L-cystine	10.0 mg

pH of the ready-to-use media at 25 °C: 7.0 ± 0.2.

5 PREPARATION

- Dissolve 23.0 g of dehydrated media (BK009) in 1010 mL of distilled or demineralized water.
- Slowly bring to boiling, stirring until complete dissolution.
- Continue boiling for 2 minutes.
- Do not autoclave.
- Cool rapidly to room temperature.
- Dispense into sterile tubes at 10 mL or in sterile vials at 100 mL.

✓ **Reconstitution:**
23.0 g/1010mL

✓ **Sterilization:**
Bring to a boil for 2 minutes

Notes:

If overheated, a brick red selenium precipitate may form, denaturing the medium, which should be discarded. Other volumes can be used depending on the protocol being followed.

6 INSTRUCTIONS FOR USE

Selective enrichment for *Salmonella* in milk and dairy products (ISO 6579-1/A1)

- Transfer 10 mL of pre-enrichment broth into 100 mL of Selenite Cystine broth.
- Incubate between 34 and 38°C for 2 successive periods of 18 to 24 hours.
- Isolate onto solid media; Brilliant Green agar and a second media of choice, with a loop of selective broth.

✓ **Inoculation:**
10 mL

✓ **Incubation:**
2 periods of 18 to 24 h between 34 and 38°C

Primary enrichment for *Salmonella* in sludge (FD/CEN/TR 15215-2)

- Transfer 10 mL of the sample suspension into 90 mL of Selenite Cystine broth.
- Transfer 1 mL and 0.1 mL of the sample suspension into tubes of 10 mL of Selenite Cystine broth.
- Incubate at 36 ± 1 °C for 22 ± 2 hours.
- Proceed with secondary enrichment by transferring 0,1 mL of the culture into tubes of Rappaport Vassiliadis broth.

✓ **Inoculation:**
10 mL, 1 mL and 0.1 mL

✓ **Incubation :**
22 h at 36 °C

Notes:

For other uses, refer to the analytical protocols in vigor.

Beyond 24 hours of incubation, there is a decreased inhibitor effect in the media. A risk exists that a powerful competitor like *Proteus* can grow at this time, which will destroy any *Salmonella* still present. On the other hand, a 48 hours incubation is required for the selective enrichment of *Salmonella Pullorum*.

7 QUALITY CONTROL

Dehydrated media: cream-white powder, free-flowing and homogeneous.

Prepared media: light amber to pinkish solution, may contain a very slight precipitate.

Typical culture response after 24 hours of incubation at 37 °C, followed by subcultures:

Microorganisms		Growth
<i>Salmonella</i> Typhimurium	WDCM 00031	> 10 characteristic colonies
+ <i>Escherichia coli</i>	WDCM 00013	
+ <i>Pseudomonas aeruginosa</i>	WDCM 00025	
<i>Salmonella</i> Enteritidis	WDCM 00030	> 10 characteristic colonies
+ <i>Escherichia coli</i>	WDCM 00013	
+ <i>Pseudomonas aeruginosa</i>	WDCM 00025	
<i>Enterococcus faecalis</i>	WDCM 00087	< 10 colonies
<i>Escherichia coli</i>	WDCM 00013	≤ 10 ² colonies

8 STORAGE / SHELF LIFE

Dehydrated base media: 2-20 °C.

The expiration date is indicated on the label.

Prepared media in tubes or vials (*) : 8 days at 2-8 °C, shielded from light and after verification of the absence of a red precipitate.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media:

500 g bottle BK009HA

10 BIBLIOGRAPHY

Leifson, E.. 1936. New selenite enrichment media for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. American Journal of Hygiene, 24 : 423-432.

FD/CEN/TR 15215-2. Avril 2006. Caractérisation des boues. Détection et dénombrement de *Salmonella* spp. dans les boues, les sols, les amendements du sol, les supports de culture et biodéchets. Partie 2 : Méthode par enrichissement en milieu liquide sélénite-cystine puis en milieu de Rapport-Vassiliadis pour la détermination semi-quantitative par la méthode du Nombre le Plus Probable (NPP).

NF U47-101. Novembre 2007. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles chez les oiseaux.

NF U47-102. Janvier 2008. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles chez les mammifères.

NF EN ISO 19250. Juin 2013. Qualité de l'eau. Recherche de *Salmonella* spp.

NF EN ISO 6579-1. Avril 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des *Salmonella* - Partie 1 : recherche des *Salmonella* spp.

NF EN ISO 6579-1/A1. March 2020. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 : detection of *Salmonella* spp. - Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC

11 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.

Document code : SELENITE CYSTINE BROTH_ENv11
Creation date : 04-2003
Updated : 06-2020
Origin of revision : Update according to the standard NF EN ISO 6579-1/A1