Product :
TRYPTONE SULFITE NEOMYCIN AGAR (TSN A)

Specification

Solid selective medium for *Clostridium perfringens* isolation.

Formula * in g/L

Casein peptone..... 15.00
Sodium sulfite.....1.00
Neomycin sulfate.....0.05
Polymyxin B..... 0.02
Yeast extract..... 10.00
Ferric citrate..... 0.50
Agar.....13.50

Final pH 7,2 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 40 g of powder in 1 L of distilled water and bring to the boil. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. For improved results, add 20 mL/L of a solution containing 1 g/L di-potassium phosphate, 0,5 g/L; sodium carbonate and 1 g/L sodium thioglycolate just before use.

Description

This culture medium was formulated taking advantage of the tolerance of *C. perfringens* to high concentrations of sulfite, which apart from being an inhibitor agent, provides a strong reducing environment.
Selection of *C. perfringens* is almost complete when it is incubated at 46°C, since neomycin and polymyxin included in the medium restrain the development of *C. bifermentans* and all the accompanying Gram negative bacteria.
The medium is especially suitable for the investigation of food products, and it may be used in tubes as well as in plates. If the incubation is not performed in an anaerobic jar, buffered thioglycolate solution must be added or the inoculated surface must be covered with a sterile layer of medium.
Colonies of *C. perfringens* form very characteristic black colonies that, if exposed to air, become decolourised by oxidation.
TSN has a very short storage period once prepared, so it is advisable to re-hydrate or reconstitute it in small amounts and use it on the day of its preparation.

Quality control

Incubation temperature: 46°C ±1,0 / ANAE **Incubation time:** 20-24 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism	Growth	Remarks
Bacillus subtillis ATCC® 6633	Inhibited	-
Clostridium perfringens ATCC® 13124	Good - very good	Black colonies
Clostridium perfringens ATCC® 10543	Good - very good	Black colonies
Escherichia coli ATCC® 8739	Inhibited	-

References

· ATLAS, R.M., & L.C. PARK (1993) Handbook of Microbiological Media, CRC Press Inc., London.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Williams & Wilkins. Baltimore, USA.
· MARSHALL, R.S., STEENBERGEN, J.F., McCLUNG, L.S. (1955) Rapid Technique for the enumeration of Clostridium perfringens. Appl. Microbiol. 13:559-563.
· MOSSEL, D.A.A. (1959) Enumeration of sulfite reducing clostridia occurring in foods. J. sci. Food Agr. 10:662-669

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).