

**Formula \* in g/L**

Meat extract.....	3,00
Peptone.....	5,00
Potassium nitrate.....	1,00
Bi-Sodium phosphate.....	2,50
Galactose.....	5,00
Agar.....	5,00

Final pH 7,3 ±0,2 at 25 °C

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

**Directions**

Suspend 21,5 g of powder in 1 L of distilled water containing 5 mL of glycerol. Bring to the boil and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

**Description**

This medium is produced according to the formulation of the US Food & Drug Administration and the ISO Standard 7937 for the identification of *Clostridium perfringens* in cosmetics and food.

**Technique**

The final containers, usually flat or standard tubes, are degassed by heating in a water bath at 100°C for 10 minutes. Then, they are cooled and they are inoculated with a needle in the middle of the medium. The inocula must be obtained from black colonies growth on TSN Agar. The inoculated tubes are incubated at 37°C for 18-20 hours without any seal or reduced atmosphere.

If the growth in this medium appears at a deep of 5-7 mm from the surface this is evidence of anaerobiosis and the absence of motility is shown by a clear and limpid growth along the streak.

For verification of nitrate reduction, several drops of a mixture of Nitrate A Reagent and Nitrate B Reagent must be added. Development of a cherry red colour shows a positive nitrate to nitrite reduction.

*Clostridium perfringens* is an anaerobic, non-motile and nitrate-reducing microorganism.

**Quality control**

**Incubation temperature:** 37°C ±1

**Incubation time:** 18-20 h

**Inoculum:** Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
<i>Clostridium perfringens</i> ATCC® 13124	Good	Nitrate (+). Nonmotile
<i>Clostridium sporogenes</i> ATCC® 11437	Good	Nitrate (-). Motile
<i>Clostridium perfringens</i> ATCC® 10543	Good	Nitrate (+). Nonmotile

**References**

- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- ISO 7937 Standard (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony count. Technique.

**Storage**

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