TSN Agar (Perfringens Selective Agar acc. to MARSHALL)

Tryptone Sulfite Neomycin Agar

Medium proposed by MOSSEL (1959) and MARSHALL et al. (1965) for the detection and enumeration of Clostridium perfringens in foodstuffs and other materials.

This highly selective culture medium provides a rapid quantitative test for Clostridium perfringens.

Mode of Action

TSN Agar (tryptone sulfite neomycin agar) exploits the high neomycin, polymyxin and sulfite tolerances and the strong sulfite-reducing power of Clostridium perfringens and the fact that this organism exhibits optimal growth at 46 °C. The growth of other sulfite-reducing clostridia is almost completely inhibited while that of the accompanying bacterial flora is largely suppressed. Addition of thioglycollate improves and stabilizes anaerobiosis.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 10.0; sodium sulfite 1.0; iron(III) citrate 0.5; Polymyxin B sulfate 0.02; neomycin sulfate 0.05; agar-agar 13.5.

Preparation

Suspend 40 g/litre, autoclave under mild conditions (10 min at 121 °C).

If required, mix in 25 ml of a filter-sterilized, buffered thioglycollate solution (4.0 % di-potassium hydrogen phosphate, 2 % sodium hydrogen carbonate, 4.0 % sodium thioglycollate) to 1 litre of the medium at a temperature of about 50 °C. Avoid subsequent heating.

pH: 7.2 ± 0.2 at 25 °C.

- **The culture medium should be prepared and use the same day.**

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

The culture medium is usually inoculated by the mixing in procedure either in test tubes or Petridishes, surface inoculation is not widely used. The thioglycollate solution described above should be added to the medium, if it is poured in Petridishes.

Incubation: at 46 °C for not longer than 18 hours, anaerobically (e.g. using Anaerocult® A, Anaerocult® A mini, or Anaerocult® P).

Clostridium perfringens forms black colonies. The plates should be inspected immediately after opening, otherwise the black colonies become paler in colour due to oxidation of the iron sulfide.

Literature

MARSHALL, R.S., STEENBERGEN, J.F., a. MCCLUNG, L.S.: Rapid technique for enumeration of Clostridium perfringens. – **Appl. Microbiol., 13**; 559-563 (1965).

MOSSEL, D.A.A.: Enumeration of sulfite reducing clostridia occuring in foods. – **J. Sci. Food Agr., 10**; 662-669 (1959).

Ordering Information

Product	Merck Cat. No.	Pack size
TSN Agar (Perfringens Selective Agar acc. to MARSHALL)	1.05264.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
di-Potassium hydrogen phosphate	1.05104.1000	1 kg
Plate basket	1.07040.0001	1 ea
Sodium hydrogen carbonate	1.06329.0500	500 g
Sodium thiglycollate	1.06691.0100	100 g

Quality control

Test strains	Growth	Black colonies
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Escherichia coli ATCC 25922	none	
Pseudomonas aeruginosa ATCC 27853	none	