

IRON SULPHITE AGAR
TM 296

For detection of thermophillic anaerobic organisms causing sulphide spoilage in foods

Composition

Ingredients	Gms/Ltr.
Agar	15.00
Casein enzymatic hydrolysate	10.00
Sodium sulphite	0.50
Ferric citrate	0.50

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for use

Dissolve 26gms in 1000ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15psi (121°C) for 15 minutes. Cool to 45-50°C and pour into sterile petri plates.

Appearance: Yellow colour, slightly opalescent gel
pH (at 25°C): 7.1 ± 0.2

Principle

IRON SULPHITE AGAR is used for detection of thermophillic anaerobic organisms causing sulphide. The medium was first developed by Wilson and Blair (1924) to enumerate *Clostridium perfringens* in water. The medium utilizes the ability of the genus *Clostridium* to reduce sulphite which reacts with the iron citrate to form ferrous sulphide, staining the colonies black. Medium contains Casein enzymatic hydrolysate as a source of nitrogen, vitamins and minerals essential for microorganisms' growth. Ferric citrate and Sodium sulphite are H₂S indicators: *Clostridium perfringens* reduces the sulphite to sulphide which in turn reacts with the iron and forms a black iron sulphide precipitate, seen as black colonies. Agar is the solidifying agent.

Interpretation

Cultural characteristics observed after inoculating (10⁴-10⁵CFU / ml), on incubating at 50°C for 24 hours, under anaerobic conditions and allow to set for 48 hours at 55°C (for thermophillic species).

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
<i>Clostridium perfringens</i>	13124	10 ⁴ -10 ⁵	Good, black colonies
<i>Clostridium sporogenes</i>	19404	10 ⁴ -10 ⁵	Good, black colonies
<i>Escherichia coli</i>	25922	10 ⁴ -10 ⁵	Inhibited

References

1. ISO 15213. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions
2. Tanner F. W., (1944). "The Microbiology of Foods", 2nd Ed., Garrard Press, Illinois, P. 1127.