

TRIPLE SUGAR IRON AGAR (as per ISO)**TM 1819**

For identification of gram negative enteric bacilli on basis of dextrose, lactose and sucrose fermentation and H₂S production

Composition

Ingredients	g/L
Agar	12.00
Lactose	10.00
Peptic digest of animal tissue	10.00
Casein enzymatic hydrolysate	10.00
Sucrose	10.00
Sodium chloride	5.00
Yeast extract	3.00
Beef extract	3.00
Dextrose	1.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024

*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 64.52gms in 1000ml purified water or distilled water. Gently heat to boil with gentle swirling and dissolve the medium completely. Distribute 10ml /culture tube or as desired. Sterilize by autoclaving at 10 psi (115°C) for 15 minutes. Cool to 45-50°C and keep in slanted position for solidification.

Appearance: Light pink colour, clear to trace hazy gel in slant position in screw cap tubes

pH (at 25°C): 7.4 ± 0.2

Principle

TRIPLE SUGAR IRON AGAR (as per ISO) is used for identification of gram negative enteric bacilli on basis of dextrose, lactose and sucrose fermentation and H₂S production.

Medium composed of Peptic digest of animal tissue, Beef extract and Yeast extract provides nitrogen, carbon and vitamins required for bacterial growth. Triple Sugar Iron Agar consists of three carbohydrates; Dextrose, Lactose and Sucrose. When carbohydrates are fermented, acid production is detected by Phenol red pH indicator.

Sodium thiosulphate is reduced to Hydrogen sulphide and Hydrogen sulphide reacts with iron salt yielding typical black iron sulphide. Ferrous sulphate is Hydrogen sulphide (H₂S) indicator and gives a typical black precipitate. Sodium chloride maintains osmotic balance of the medium. Agar is used as a solidifying agent.



PRODUCT DATA SHEET

Microbiological parameters (Growth promotion test)

Culture characteristics observed after inoculation (10^3 CFU/ml) and incubation at 35 - 37°C for 18 – 24 hours.

Test strains	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt / Gas	H ₂ S
<i>Salmonella typhimurium</i>	14028	10^3	Luxuriant	Alkaline, red	Acidic, yellow/+ ve	+ ve
<i>Escherichia coli</i>	25922	10^3	Luxuriant	Acidic, yellow	Acidic, yellow/ + ve	- ve
<i>Shigella flexneri</i>	12022	10^3	Luxuriant	Alkaline, red	Acidic, yellow/- ve	- ve
<i>Proteus vulgaris</i>	13315	10^3	Luxuriant	Alkaline, red	Acidic, yellow/- ve	+ ve

Alkaline; Colour changes to red.

Acidic; Colour changes to yellow.

H₂S production; Blackening of medium.

+ ; positive reaction

- ; negative reaction

References

1. Russell, F. F. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. 25:217. (1911).
2. Kligler, I. J. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health 7:1042-1044. (1917).
3. Kligler, I. J. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. 28:319-322. (1918).
4. Sulkin, S. E., and J. C. Willett. A triple sugar-ferrous sulphate medium for use in identification of enteric organisms. J. Lab. Clin. Med. 25:649-653. (1940).
5. ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp Standard Methods for the Examination of Dairy Products. APHA, (1972).