IRON SULFITE AGAR ISO 15213

CAT Nº: 1559

For the enumeration of sulfite – reducing bacteria growing under anaerobic conditions

FORMULA IN g/l

Enzymatic Digest of Casein	15.00	Ferric Ammonium Citrate	1.00
Soy Peptone	5.00	Sodium Disulfite	1.00
Yeast Extract	5.00	Bacteriological Agar	13.50

Final pH 7.6 ± 0.2 at 25ºC

PREPARATION

Suspend 40.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute or until complete dissolution. Distribute in flasks and sterilize in autoclave at 121ºC for 15 minutes. The prepared medium should be stored at 2-8ºC. The color of the prepared medium is amber slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

IRON SULFITE AGAR is recommended by ISO 15213 for the enumeration of sulphite reducing bacteria.

Enzymatic Digest of Casein and Soy Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group essential for bacterial growth. Ferric citrate and Sodium disulfite are H_2S indicators: *Clostridium perfringens* reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Bacteriological agar is the solidifying agent.

This medium can be used in tube or plate. When used in plates, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample if the product is liquid, or 1 ml of the first decimal dilution of the initial suspension (10^{-2}) in the case of other products. Pour into each Petri dish approximately 15 ml of iron sulfite agar. Add the inoculum to the medium, mix it carefully and allow the medium to solified. After the medium has solidified, pour 5 ml to 10ml of the same medium into the dish as an overlay. If tubes are used, inoculate 1ml from each dilution into each of two tubes of medium. Mix gently and leave the medium to solidify. After the medium has solidified, pour 2 to 3ml of the same medium into each tube as an overlay.

After solidification, incubate the Petri dishes in anaerobic jars at 37ºC±1ºC for 24-48 hours. If thermophilic bacteria are suspected, incubate at 50±1ºC.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 37± 1°C, under anaerobic conditions, and observed after 24-48 hours (final reading after 48 hours) under anaerobic conditions.

According to ISO 11133 : 24±3-48±2 h/37± 1°C (anaerobic atm.) (Productivity and Specificity)

Microorganisms	Inoculum (cfu)	Reference media	Productivity Quantitative	Specificity Qualitative	Characteristic reaction
Clostridium perfringens ATCC 13124	10^2	TSA or other nonselective medium for anaerobes	$P_R \geq 0.5$		Black colonies
Clostridium perfringens ATCC 12916	10^2	TSA or other nonselective medium for anaerobes	$P_R \geq 0.5$		Black colonies
Escherichia coli ATCC 25922	10^3- 10^4			No blackering	

BIBLIOGRAPHY

ISO 15213. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions

STORAGE

Once opened keep powdered medium closed to avoid hydration.