Iron Sulphite Agar Modified

M1852I

Iron Sulphite Agar Modified is used for the enumeration of sulphite-reducing bacteria growing under anaerobic conditions.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of casein	15.000
Pancreatic digest of soyabean meal	5.000
Yeast extract	5.000
Disodium disulfite	1.000
Iron (III) Ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired.

Principle And Interpretation

Iron Sulphite Agar, Modified is recommended by ISO for the enumeration of sulphite reducing bacteria.(1). Most Clostridia possess sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Enzymatic Digest of Casein and pancreatic digest of soyabean meal provide nitrogen, vitamins, minerals and amino acids necessary for the growth of organism. Yeast extract serves as a rich reservoir of vitamins especially B-complex vitamins. Ferric citrate ammonium citrate and Disodium sulfite serves as are H2S indicators, wherein *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. Agar is the solidifying agent.

Enumeration with this medium can be performed using either tubes or plates. In case of tubes distribute 20-25 ml of the medium in tubes and inoculate 1 ml of test sample or 1 ml of serial dilutions of 10-1 and 10-2 in molten state. Allow to solidify , and pour 2-3 ml of the same medium in each tube to overlay. In case of Petri plates, transfer 1 ml of test sample or initial dilution. Further dilution can be carried out and 1 ml of each dilution (10-1 and 10-2) is transferred to an empty Petri plate. Cool the medium to 44-47°C and pour 15-20 ml of the medium to the Petri plate containing the inoculum. Mix the inoculum and allow the medium to solidify. Overlay the medium with 5-10 ml of the same medium.

After solidification , incubate the medium at 36-38°C for 24-48 hours. If thermophilic bacteria are suspected, a second of tubes is incubated at 49-51°C for 24-48 hours. After incubation, black coloured colonies, possibly surrounded by a black zone are counted as sulphite reducing bacteria.

Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed under anaerobic conditions, after an incubation at 36-38°C for 24-48 hours.

Cultural Response

Organism	Inoculum	Growth	Recovery	Colour of colony
Cultural Response				
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	black
Clostridium butyricum ATCC 13732	50-100	luxuriant	>=50%	black
Clostridium sporogenes ATCC 19404	50-100	luxuriant	>=50%	black
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
Escherichia coli ATCC 25922	50-100	good	40-50%	no blackening

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

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

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