Iron Sulfite Agar ISO

Cat. 1559

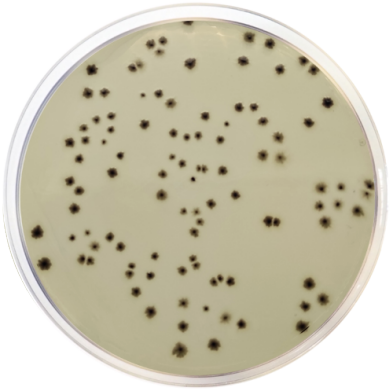
For the enumeration of sulfite – reducing bacteria growing under anaerobic conditions.

Practical information

Aplications	Categories
Selective enumeration	Sulfite–reducing bacteria

Industry: Food

Regulations: ISO 11133 / ISO 15213



Principles and uses

Iron Sulfite Agar is recommended by the normative ISO 15213 for the enumeration of sulphite-reducing bacteria and ISO 6461 for the detection and enumeration of the spores of sulfite-reducing anaerobes (Clostridia).

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 H2S 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.

Formula in g/L

Enzymatic digest of casein	15	Bacteriological agar	13,5
Ferric ammonium citrate	1	Soy peptone	5
Yeast extract	5	Sodium disulfite	0,5

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 40 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.

Instructions for use

Enumeration of sulfite-reducing bacteria growing under anaerobic conditions according to ISO 15213:
- Take plates or tubes to prepare the Iron Sulfite medium.
- If plates are used, 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.
- 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.
- Incubate the plates or tubes in anaerobic jars at 37±1 ºC for 24-48 hours.
- If thermophilic bacteria are suspected, incubate at 50±1 ºC.

Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) by the membrane filtration method according to ISO 6461:
- Apply heat to the sample for a period of time sufficient to destroy the vegetative bacteria (75±5 ºC for 15 minutes).
- Filtrate the water sample through a membrane filter

- Place the filter on the Iron Sulfite Agar.
- Incubate at 37±1 ºC for 20±4 hours and 44±4 hours in anaerobic conditions.
- Count all black colonies.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber slightly opalescent	7,6 ± 0,2

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity quantitative (37±1 ºC / 24±3 - 48±2 h) / Productivity qualitative (37±1 ºC / 24±3 - 48±2 h) / Specificity (37±1 ºC / 24±3 - 48±2 h). Anaerobic atmosphere.
Inoculation conditions: Productivity quantitative (100±20. Min. 50 cfu) / Specificity (10^3 - 10^4 cfu)
Reference media: TSA

Microorganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 12916	Good growth, >50%	Black colonies
Clostridium perfringens ATCC 13124	Good growth, >50%	Black colonies
Escherichia coli ATCC 25922	Growth	No blackening

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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