

Iron Sulphite agar

Iron Sulphite Agar is recommended for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

REF: BS.1/IS01.100.0100	100 Gram	REF: BS.1/IS01.250.0250	250 Gram
REF: BS.1/IS01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America (1). It was shown by Beerens (2) that 0.1% sulphite concentration in the original formula was inhibitory to some strains of *Clostridium sporogenes*. This observation was later confirmed by Mossel et al (3), who consequently showed that 0.05% sulphite concentration was not inhibitory to the organisms. Most clostridia have 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.

METHOD PRINCIPLE

Casein enzymic hydrolysate provides nitrogen and other nutrients necessary to support bacterial growth. Sulphite-reducing bacteria usually produce black colonies as a result of the reduction of sulphite to sulphide, which reacts with the iron (III) salt. For the detection of organisms causing sulphide spoilage, two methods can be followed: a) Deep-Shake Culture Method: Dispense the medium in 10 ml amounts in tubes. Inoculate the sample when the medium is at about 50°C. Allow to set and incubate at 55°C for 24-48 hours. Typical thermophilic species - *Desulfotomaculum nigrificans*, produces distinct black spherical colonies in the depth of the medium. b) Attenborough and Scarr (4) Method: In this method, diluted samples of sugar or any other food are filtered through membrane filters. These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them. The medium is allowed to set and then incubated at 55-56°C for 24-48 hours. After incubation, the number of black colonies on the membrane filter is counted. Confirmation tests are further carried out to identify the organism growing in the medium. This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples. The blackening reaction is only presumptive evidence of clostridial growth. Confirmation test must be carried out for identification. There are many gram-negative bacteria that are able to reduce sulfite with iron sulfide production in this medium, but in these cases the enzymes are extra cellular and the entire medium becomes dark, rendering their enumeration impossible.

MEDIA COMPOSITION

Item	Formula per liter of medium
- Casein enzymic hydrolysate	10
- Sodium sulphite	0.5
- Ferric citrate	0.5
- Agar	15

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.

- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental Contamination.

S61: avoid release in environment.

For further information, refer to the Iron Sulphite Agar material safety data sheet.

STORAGE AND STABILITY

BioScien Iron Sulphite Agar dehydrated media are stable until expiration date stated on label when properly stored 10-30°C. The prepared medium should be stored at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 7.1 ± 0.2 at 25°C

MEDIA PREPARATION

1. Suspend 26 grams in 1000 ml distilled water.
2. Adjust pH to 7.1 ± 0.2 at 25°C
3. Heat to boiling to dissolve the medium completely.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
5. Mix well and pour into sterile Petri plates.

Deterioration

The color of **BioScien** Iron Sulphite Agar Light yellow to brownish yellow homogeneous free flowing powder. If there are any physical changes, discard the medium.

The hydrated medium is Yellow coloured, slightly opalescent gel forms in Petri plates, media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

SPECIMEN COLLECTION AND PRESERVATION

Food samples; Water and sewage samples

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile plates
- Sterile loops
- Incubator

PERFORMANCE CHARACTERISTICS

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Fits your perfection

Cultural characteristics observed after an incubation at 30°C for 40-48 hours.

Test Organisms	Growth	Colony Color
<i>Clostridium botulinum</i> ATCC 25763	Good - Luxuriant	Black
<i>Clostridium</i> <i>butyricum</i> ATCC 13732	Good - Luxuriant	Black
<i>Clostridium</i> <i>sporogenes</i> ATCC 19404	Good - Luxuriant	Black
<i>Desulfotomaculum</i> <i>nigrificans</i> ATCC 19998	Good - Luxuriant	Black
<i>Escherichia coli</i> ATCC 25922	Good	No blacking

SYMBOLS IN PRODUCT LABELLING		
IVD	For in-vitro diagnostic use	 Number of <n> test in the pack
LOT	Batch Code/Lot number	 Caution
REF	Catalogue Number	 Do not use if package is damaged
	Temperature Limitation	 Consult Instruction for use
	Expiration Date	
	Manufactured by	

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact **BioScien** technical support.

REFERENCES

- 1.Tanner F. W., 1944, "The Microbiology of Foods", 2nd Ed., Garrard Press, Illinois, P. 1127.
- 2.Beerens H., 1958, DSIR, Proc. 2nd Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.
- 3.Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S., 1959, J. Path. Bacteriol., 78:290.
- 4.Attenborough J. and Scarr M., 1957, J. Appl. Bacteriol., 20: 460.



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