PRODUCT SPECIFICATION SHEET

Iron Sulphite Agar (DM122)

Intended Use
Iron Sulphite Agar (DM122) is recommended for detection of thermophilic anaerobic organisms causing sulphide spoilage in foods.

Product Summary and Explanation
Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America.[1] It has a reduced concentration of sodium sulphite to allow improved detection of some strains of *Clostridium sporogenes*. Beerens[2] demonstrated 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 further observed 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 H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Principles of the Procedure
Iron Sulphite Agar contains casein enzymic hydrolysate provides nitrogen, vitamins, amino acids and other nutrients necessary to support bacterial growth. Ferric citrate and Sodium disulfite are H_2S indicators. Sulphite is reduced to sulphide by sulphite reducing organisms, which reacts with the iron (III) salt to yield a black precipitate.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Sodium sulphite	0.50
Iron (III) citrate	0.50
Agar	15.00
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 26 grams in one liter distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to brownish yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured, slightly opalescent gel forms in Petri plates
Reaction of 2.6% Solution	pH : 7.1 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed under anaerobic conditions, after an incubation at 55-56°C for 24-48 hours.

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Sr. No.	Organisms	Results to be achieved			
		Inoculum	Growth	Recovery	Colour of Colony
1.	*Clostridium botulinum ATCC 25763*	50-100	good-luxuriant	>=50%	black
2.	*Clostridium butyricum ATCC 13732*	50-100	good-luxuriant	>=50%	black
3.	*Clostridium sporogenes ATCC 19404*	50-100	good-luxuriant	>=50%	black
4.	*Desulfotomaculum nigrificans ATCC 19998*	50-100	good-luxuriant	>=50%	black
5.	*Escherichia coli ATCC 25922*	50-100	Good	40-50%	no blackening

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Refer to appropriate references for standard test procedures.
2. For the detection of organisms causing sulphide spoilage, two methods can be followed:
   a) Deep-Shake Culture Method:
      1. Dispense the medium in 10 ml amounts in tubes.
      2. Inoculate the sample when the medium is at about 50°C.
      3. Allow to set and incubate at 55°C for 24-48 hours.
   b) Attenborough and Scarr Method:
      1. Diluted samples of sugar or any other food are filtered through membrane filters.
      2. These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them.
      3. Allow to set and then incubated at 55-56°C for 24-48 hours.

Results

a) Deep-Shake Culture Method
Typical thermophilic species, e.g. Desulfotomaculum nigrificans , produce distinct black spherical colonies in the depth of the medium.

b) Attenborough and Scarr Method
Count the number of black colonies on the membrane filter. Confirmation tests should be carried out to identify the organism growing in the medium. This technique is quicker, of comparable accuracy and permits the examination of larger samples.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The blackening reaction is only presumptive evidence of clostridial growth. Confirmation test must be carried out for identification.
2. 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.
3. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.

4. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Iron Sulphite Agar
Product Code : DM122
Available Pack sizes : 100gm/ 500gm

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.

Further Information

For further information please contact your local MICROMASTER Representative.

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