# Iron Sulphite Agar (NCM0221)

**Intended Use**

Iron Sulphite Agar is a medium for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food, and is not intended for use in the diagnosis of disease or other conditions in humans.

**Description**

This formulation is a modification of Cameron Sulphite Agar, which was developed by the National Canners Association of America (now the Grocery Manufacturers Association). Iron Sulphite Agar has a reduced concentration of sodium sulphite to allow improved detection of some strains of *Clostridium sporogenes*. Beerens, and later Mossel, demonstrated that some strains of *C. sporogenes* would not tolerate sodium sulphite levels of 0.1%. Mossel further observed that reducing sulphite content to 0.05% improved detection of these strains.

Tryptone provides nitrogen and other nutrients necessary to support bacterial growth. The presence of sulphite reducing bacteria is indicated by the formation of black colonies. These colonies form when bacteria reduce sulphite to sulphide, which reacts with iron (III) citrate to yield a black precipitate.

**Typical Formulation**

| | |
|---|---|
| Tryptone | 10.0 g/L |
| Sodium Sulphite | 0.5 g/L |
| Iron (III) Citrate | 0.5 g/L |
| Agar | 12.0 g/L |

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

**Precaution**

Refer to SDS

**Preparation**

1. Suspend 23 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.

**Test Procedure**

Use ‘deep shake’ or ‘Attenborough and Scarr overlay’ methods for inoculation.

Deep-Shake Culture Method

Dispense the medium in 10mL volumes in tubes. Inoculate the sample when the medium is at approximately 45-50°C. Allow to set.

Attenborough and Scarr Method

This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples.

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 45-50°C) to cover them. The medium is allowed to set.

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Incubation
Incubate for 24-48 hours at 55°C for thermophilic organisms or 37°C for mesophilic organisms. May also be used for mesophilic sulphite reducers if incubated at 37°C.

**Quality Control Specifications**
**Dehydrated Appearance:** Powder is homogeneous, free flowing and beige with brown flecks.

**Prepared Appearance:** Prepared medium is a clear light tan gel.

**Minimum QC:**
*Desulfotomaculum nigrificans* ATCC 7946
*Clostridium sporogenes* ATCC 19404
*Escherichia coli* ATCC 25922

**Results**
Refer to appropriate references for results.

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

**Limitations of the Procedures**
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

**Storage**
Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

**References**
1. Attenborough, S.J. & Scarr, P.M. (1957). *J. Appl. Bact.* 20. p460-466.
2. Beerens, H. (1958) DSIR. Proc. 2nd Internat. Symp. Food. Microbiol. 1957, HMSO, London, pp. 235-245.
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4. Mossel, D.A.A., Golstein Brouwers, G.W.M.V. & de Bruin, A.S. (1959). *J. Path. Bact.* 78. 290-291.
5. Tanner, F.W. (1944). The Microbiology of Foods, 2nd edition, Garrard press, Illinois, p1127.

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