Iron Sulphite Agar, Granulated

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

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>0.500</td>
</tr>
<tr>
<td>Iron (III) citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

Suspend 26 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile tubes or Petri plates as desired.

**Principle And Interpretation**

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.

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 45-50°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 45-50°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.

**Quality Control**

**Appearance**

Light yellow to brownish yellow coloured granular medium

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Please refer disclaimer Overleaf.
Yellow coloured, slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.1±0.2

**pH**

6.90-7.30

**Cultural Response**

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

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium botulinum ATCC 25763</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>Clostridium butyricum ATCC 13732</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>Clostridium sporogenes ATCC 19404</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>Desulfotomaculum nigrificans ATCC 19998</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>50-100</td>
<td>good</td>
<td>40-50%</td>
<td>no blackening</td>
</tr>
</tbody>
</table>

**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**


Revision : 00 / 2014

**Disclaimer**

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