TECHNICAL DATA SHEET

IRON SULFITE AGAR (TSC AGAR BASE)

ENUMERATION OF SULFITE-REDUCING ANAEROBES

1 INTENDED USE

Iron sulfite agar is a media used for the enumeration of sulfite-reducing anaerobes in food microbiology but also in water.

The typical composition corresponds to that defined in the standards NF EN 26461-2 and NF ISO 15213.

The agar base can also be used with Cycloserine for the more specific detection of *Clostridium perfringens* (refer to the technical data sheet for TSC Agar).

2 PRINCIPLES

Sulfur reducing microorganisms reduce the sodium sulfite to sulfide, which with ferric citrate forms a black iron sulfide precipitate around the colonies.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 15,0 g
- Papaic digest of soybean meal ..... 5,0 g
- Yeast extract ..... 5,0 g
- Sodium metabisulfite ..... 1,0 g
- Ferric ammonium citrate ..... 1,0 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25°C : 7,6 ± 0,2.

4 PREPARATION

Preparation from dehydrated media :

- Dissolve 42,0 g of dehydrated media (BK031) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 20 mL in 20 x 200 mm tubes or 100 mL per flask.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution : 42,0 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of ready-to-melt media in vials or in tubes :

- If prepared in advance as above, or if using the ready-to-melt media (BM039 or BM077), melt the agar for the minimum amount of time necessary to achieve complete liquefaction.
- Cool and maintain the media in a molten state at 44-47 °C.

5 INSTRUCTIONS FOR USE

Food microbiology, enumeration of sulfur reducing anaerobic bacteria (NF ISO 15213)

- The inoculation can be performed in 20 mL tubes or in Petri plates, at the users convenience.
- Heat, if necessary the product to test in order to destroy vegetative cells and activate the spores.

In tubes

- Into each 20 mL of molten media maintained at 44-47°C, transfer 1 mL if inoculum and its serial dilutions into the corresponding number of tubes, while avoiding the incorporation of air into the media.
- Mix well.
- Cool in an ice water bath.
- Incubate at 37 ± 1 °C for 24 and 48 hours.

✓ **Inoculation :**
In tubes in depth

✓ **Incubation :**
24 h and 48 h at 37 °C

In Petri plates

- Transfer 1 mL of inoculum and its serial dilutions to empty, sterile Petri plates.
- Pour 15 to 20 mL of base media brought to 44-47 °C
- Homogenize well.
- Let solidify on a flat surface.
- Add a second layer of agar and let solidify.
- Incubate the plates in an anaerobic jar for 24 and 48 hours at 37 °C in a mixed atmosphere of hydrogen and carbon dioxide.

✓ **Inoculation :**
1 mL in a double layer

✓ **Incubation :**
Anaerobic conditions
24 and 48 h at 37 °C

Water quality, enumeration of spores of anaerobic sulfer reducing bacteria (NF EN ISO 26461-2)

- Maintain the base media at 44-47 °C.
- Heat the inoculum in order to destroy the vegetative forms and activate the spores.
- Filter a known quantity of water through a nitrocellulose membrane.
- Deposit the membrane, filtered side down and making sure that the membrane and agar are in close contact, with no trapped air bubbles.
- Quickly pour a second layer, in order to obtain a thickness of 5mm total of agar.
- Let solidify on a flat, cool surface.
- Incubate under anaerobic conditions for 24 ± 4 and 44 ± 4 hours at 37 °C.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
Anaerobic conditions
20 to 48 h at 37 °C

NOTE
It is also possible to place the filter on the surface of the agar, filtered side up.

6 RESULTS

Count colonies surrounded by a black halo.
Note the results upon opening the anaerobic jar, as the colonies risk to become pale and dull due to oxidation upon exposure to air.
Because of the confluence of halos, it is often necessary to make intermediate counts.

See ANNEX 1 : PHOTO SUPPORT .

7 QUALITY CONTROL

Dehydrated base media : beige powder, free-flowing and homogeneous.
Prepared media (ready-to-melt) : amber agar.

Typical culture response after 24-48 hours of incubation at 37 °C, in 90mm plates (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio)	Characteristic colonies
Clostridium perfringens	WDCM 00007	$P_R \geq 50$ %	Black
Escherichia coli	WDCM 00013	Inhibited, score 0	-

8 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Ready-to-melt media : 2-25 °C.
The expiration dates are indicated on the label.

Prepared base media in vials or tubes (*) : 180 days at 2-25 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media :
500 g bottle BK031HA

Ready-to-melt media :
10 x 200 mL vials BM07708
50 x 20 mL tubes BM03908

10 BIBLIOGRAPHY

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NF EN 26461-2. Juillet 1993. Qualité de l'eau. Recherche et dénombrement des spores de micro-organismes anaérobies sulfito-réducteurs (*clostridia*). Partie 2 : Méthode par filtration sur membrane.

NF ISO 15213. Septembre 2003. Microbiologie des aliments. Méthode horizontale pour le dénombrement des bactéries sulfito-réductrices se développant en conditions anaérobies.

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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ANNEX 1 : PHOTO SUPPORT

IRON SULFITE Agar

Detection and enumeration of anaerobic sulfur-reducing bacteria

Results :

Growth obtained after 24 hours of incubation at 37 °C, under anaerobic conditions.