

# Sulfite Iron Agar, Base

For the detection and enumeration of clostridia in meat and meat products.

This culture medium complies with the recommendations of the International Organization for Standardization (ISO) (1971).

## Mode of Action

Brief heating (1 min at 80 °C) kills the vegetative cells present in the sample material whilst the bacterial spores survive and germinate. The H<sub>2</sub>S-positive ones reduce the sulfite in the culture medium to sulfide, which reacts with iron to form black iron sulfide. This stains the concerning colonies black and the H<sub>2</sub>S-weakly-positives brown. In an anaerobic environment clostridia grow to form black colonies under these conditions.

## Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 10.0; sodium sulfite 0.5; agar-agar 15.0.

### Also to be added:

Iron(II) sulfate 1.4.

## Preparation

Suspend 40.5 g/litre, if desired dispense into small flasks, autoclave (15 min at 121 °C); at about 50 °C add 20 ml of a 7% iron(II) sulfate solution/litre, mix and pour plates.

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and yellowish to yellowish-green.

## Experimental Procedure and Evaluation

Preparation of the sample: in accordance with the ISO recommendations, homogenize the comminuted sample with 9 times its own weight of sterile diluent solution (peptone from casein 0.1 %, cysteinium chloride 0.05 %, sodium chloride 0.85%). Dispense 50 ml aliquots into 100ml flasks and heat for 1 minute at 80°C in a water bath. Cool immediately in cold water.

Spread the sample material thinly on duplicate Sulfite Iron Agar plates. Incubate one plate aerobically and the other anaerobically for 2 days at 35 °C. Mesophilic clostridia are present, if

- only the anaerobically incubated plates show blackening and
- a catalase test using Bactident® Catalase performed on these plates is negative.  
Gas formation: culture is catalase-positive.  
No gas formation: culture is catalase-negative.

**Note: when using vented Petridishes, the medium needs over-layer and anaerobic incubation to get black colonies.**

**Alternative in tubes: mixing in method, aerobic incubation**

## Literature

International Organization for Standardization (ISO): Meat and Meat Products. - Mesophilic Clostridial Spores - Working Draft ISO/TC/34/SC6 (1971).

## Ordering Information

Product	Merck Cat. No.	Pack size
Sulfite Iron Agar, Base	1.10864.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® a mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Bactident® Catalase	1.11351.0001	1 x 30 ml
Iron(II)sulfate heptahydrate	1.03965.0100	100 g
L-Cysteinium chloride monohydrate	1.02839.0025	25 g
Peptone from casein	1.07213.1000	1 kg
Plate basket	1.07040.0001	1 ea
Sodium chloride purified	1.06400.1000	1 kg

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## Quality control

Test strains	Growth	Black colonies
<i>Clostridium perfringens</i> ATCC 10543	good / very good	+
<i>Clostridium perfringens</i> ATCC 13124	good / very good	+
<i>Clostridium botulinum</i>	good / very good	+
<i>Clostridium tetani</i> ATCC 19406	good / very good	+
<i>Escherichia coli</i> ATCC 25922	fair / very good	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	poor / good	-
<i>Bacillus cereus</i> ATCC 11778	poor / good	-



*Bacillus cereus*  
ATCC 11778



*Clostridium botulinum*



*Clostridium perfringens*  
ATCC 13124



*Clostridium tetani*  
ATCC 19406