Instructions For Use
ENGLISH

Iron Sulphite Agar

Medium for detection and enumeration of sulphite-reducing *Clostridium* spp., according to ISO 15213-1.

DESCRIPTION

Iron Sulphite Agar is a medium used for the detection of sulphite-reducing *Clostridium* species by colony-count technique.

This medium complies with ISO 15213-1 and is intended for the examination of foods, animal feeding stuffs and environmental samples in the area of food production and handling.

TYPICAL FORMULA*	(g/l)
Enzymatic Digest of Casein	15.0
Enzymatic Digest of Soya	5.0
Yeast Extract	5.0
Sodium Disulfite (sodium metabisulfite) anhydrous	0.5
Iron(III) Ammonium Citrate	1.0
Agar	15.0

Final pH 7.6 ± 0.2 at 25°C

*Adjusted and/or supplemented as required to meet performance specifications;
Grams per litre of purified water.

METHOD PRINCIPLE

Enzymatic digest of casein and pancreatic digest of soya provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a rich source of vitamins of B-group. Ferric ammonium citrate and sodium metabisulfite are H_2S indicators. Agar is the solidifying agent. *Clostridia* reduce sulfite to sulfide which reacts with iron to form a black iron sulfide precipitate.

PREPARATION

Dehydrated culture medium (DCM)

Suspend 41.5 g of the powder in 1 liter of distilled or deionized water. Heat shaking frequently until completely dissolved. Autoclave at 121°C for 15 minutes.

Medium in bottles

Melt the content of a bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool to 45-50°C, mix well avoiding foam formation before pouring.

TEST PROCEDURE

Following ISO 15213-1, inoculate Iron Sulphite Agar (ISA) using the pour plate method: Transfer 1 ml test sample or 1 ml initial suspension to duplicate plates, pour the medium (12-15 ml for 90 mm Petri dishes or 45-50 ml for 140 mm Petri dishes) molten and tempered at 44-47°C into each Petri dish. Carefully mix the inoculum with the medium. Repeat the procedure with further dilutions. After the agar has solidified, pour the ISA medium (5 ml for 90 mm Petri dishes or 10 ml for 140 mm Petri dishes) as overlay.

If the scope of the test is to count only spores, heat the decimal dilution series to 80°C in a water bath for 10 ± 1 min.

If 60 mm ready-to-use plates are used, inoculate the medium with the membrane filter technique.

Incubate the inoculated plates at 37 ± 1°C for 48 ± 2 hours under anaerobic atmosphere.

INTERPRETING RESULTS

Count all black or grey to yellow-brown colonies as presumptive sulphite-reducing *Clostridium* spp.

Confirmation suspect colonies by subculturing onto two non-selective blood agar plates (e.g. Columbia blood agar) or another nutrient-rich medium (e.g. Tryptone soya agar or Brain heart infusion agar). From each pair of plates, one is incubated aerobically and the other anaerobically at 37°C for 20 ± 2 h. Colonies belonging to the genus *Clostridia* will grow only on the agar plate incubated in an anaerobic atmosphere.

NOTE When no confirmation is performed, the results can be reported as “anaerobic sulphite-reducing bacteria”.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store prepared medium in bottles and plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 1 year.
Ready-to-use plates: 4 months.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige.

Appearance of Prepared Medium: Slightly opalescent, light amber.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Clostridium perfringens	WDCM 00007 (ATCC 13124; NCTC 8237)	50-100 CFU	48 ± 2 h/ 37 ± 1°C anaerobic atmosphere	Good growth, black colonies ($P_R \geq 0.5$ on TSA)
Escherichia coli	WDCM 00012 (ATCC 8739; NCTC 12923)	10^3-10^4 CFU		Weak to good growth, no blackening of colonies

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Iron Sulphite Agar ISO 15213-1	60 mm agar Plate	20 plates	163372
	Bottle	6 x 100 ml	403180
	Dehydrated Culture Medium	100 g	621401
	Dehydrated Culture Medium	500 g	611401