TM 296 - IRON SULPHITE AGAR

INTENDED USE

For detection of thermophillic anaerobic organisms causing sulphide spoilage in foods.

PRODUCT SUMMARY AND EXPLANATION

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America. It was shown by Beerens 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, 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 H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

For the detection of organisms causing sulphide spoilage, two methods can be followed:

Deep-Shake Culture Method: Dispense the medium in 10 ml amounts in tubes. Inoculate the sample when the medium is at about 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.

Attenborough and Scarr 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 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 clostridia 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.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	10.000
Sodium sulphite	0.500
Iron (III) citrate	0.500
Agar	15.000

PRINCIPLE

Tryptone 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.

INSTRUCTION FOR USE

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

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to brownish yellow homogeneous free flowing powder.
Appearance of prepared medium : Yellow coloured, slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.1±0.2

INTERPRETATION

Cultural characteristics observed under anaerobic conditions, after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Clostridium botulinum	25763	50-100	Luxuriant	>=70%	55-56°C	24-48 Hours
Clostridium butyricum	13732	50-100	Luxuriant	>=70%	55-56°C	24-48 Hours
Clostridium sporogenes	19404	50-100	Luxuriant	>=70%	55-56°C	24-48 Hours
Desulfotomaculum nigrificans	19998	50-100	Luxuriant	>=70%	55-56°C	24-48 Hours
Escherichia coli	25922	50-100	Good	40-50%	55-56°C	24-48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

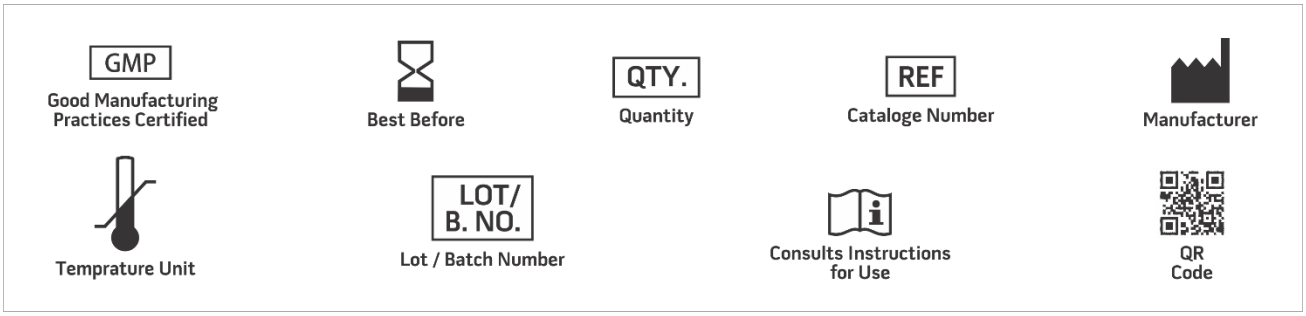
DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Attenborough J. and Scarr M., 1957, J. Appl. Bacteriol., 20: 460.
2. Beerens H., 1958, DSIR, Proc. 2nd Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S., 1959, J. Path. Bacteriol., 78:290.
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Tanner F. W., 1944, "The Microbiology of Foods", 2nd Ed., Garrard Press, Illinois, P. 1127.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

Revision: 08 Nov., 2019