

TM 577 – REINFORCED CLOSTRIDIAL AGAR

INTENDED USE

For cultivation and enumeration of Clostridia and other anaerobes.

PRODUCT SUMMARY AND EXPLANATION

Hirsch and Grinstead formulated Semisolid Reinforced Clostridial Medium and found that clostridia from small amount of samples could be grown well on this medium with higher viable cell counts. This medium can also be used for growing anaerobic and facultative bacteria. Barnes et al used a solid (agar) version of the medium to develop vegetative cells in assays of *Clostridium perfringens*. Reinforced Clostridial Medium was used in the enumeration of clostridia from food.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	10.000
Beef extract	10.000
Yeast extract	3.000
Dextrose (Glucose)	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Starch, soluble	1.000
L-Cysteine hydrochloride	0.500
Agar	13.500

PRINCIPLE

This medium consists of Typtone and Beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic byproducts. Cysteine hydrochloride is the reducing agent. Sodium acetate acts as a buffer. This medium can be made selective by addition of 15-20 mg polymyxin B per litre of media.

INSTRUCTION FOR USE

- Dissolve 51.0 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 dispense as desired.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder** : Cream to yellow homogeneous free flowing powder.
- Appearance of prepared medium** : Light yellow coloured clear to slightly opalescent gel forms in Petri plates.
- pH (at 25°C)** : 6.8 ± 0.2



INTERPRETATION

Cultural characteristics observed in an anaerobic atmosphere after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
<i>Bacteroides fragilis</i>	23745	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours
<i>Bacteroides vulgatus</i>	8482	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours
<i>Clostridium butyricum</i>	13732	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours
<i>Clostridium perfringens</i>	13124	50-100	Good-luxuriant	>=50%	35-37°C	40-48 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. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Barnes E. M., Despaul J. E. and Ingram M., 1963. J. Appl. Bacteriol. 26:41
3. Barnes E. M. and Ingram J. E., 1956. J. Appl. Bacteriol. 19:11
4. Hirsch A. and Grinstead C., 1954, J. Dairy Res. 21:10 Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.



 GMP Good Manufacturing Practices Certified	 Best Before	 Quantity	 Catalogue Number	 Manufacturer
 Temperature Unit	 Lot / Batch Number	 Consults Instructions for Use	 QR Code	

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

***For Lab Use Only**
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