

Reinforced Clostridial Agar

Intended Use

Reinforced Clostridial Agar is used for the cultivation and enumeration of Clostridia and other anaerobes.

Summary

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.

Principle

Reinforced Clostridial Agar contains casein enzymic hydrolysate 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 liter of media.

Formula*

Ingredients	g/L
Beef extract	10.0
Casein Enzymic Hydrolysate	10.0
Sodium Chloride	5.0
Dextrose	5.0
Sodium Acetate	3.0
Yeast Extract	3.0
Starch soluble	1.0
L-Cysteine Hydrochloride	0.5
Agar	13.5
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen

Food and Dairy samples.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 51.00 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance: Light yellow coloured, slightly opalescent gel forms in petridishes.

Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 40-48 hours under anaerobic condition.

Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time, inoculating ≤100 cfu of appropriate microorganism.

Organism (ATCC)	Growth
<i>Bacteroides vulgatus</i> (8482)	Good
<i>Bacteroides fragilis</i> (1045)	Good
<i>Clostridium perfringens</i> (13124)	Good

Note: For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Hirsch A. and Grinstead C., 1954, J. Dairy Res. 21:101.
2. Barnes E. M., Despaul J. E. and Ingram M., 1963. J. Appl. Bacteriol. 26:415.
3. Barnes E. M. and Ingram J. E., 1956. J. Appl. Bacteriol. 19:117.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201180080100	Dehydrated Culture Media	100 g
201180080500	Dehydrated Culture Media	500 g
205180270100	Ready Prepared Plate (90 mm)	100 Plates

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
