



PRODUCT SPECIFICATION SHEET

Reinforced Clostridial Agar (DM223)

Intended Use

Reinforced Clostridial Agar (DM223) is recommended for the cultivation and enumeration of *Clostridia* and other anaerobes.

Product Summary and Explanation

Hirsch and Grinstead formulated semisolid Reinforced Clostridial Medium and found that the medium outperformed other media in supporting growth of clostridia from small amount of inocula and produced higher viable cell counts.⁽¹⁾ This medium can also be used for growing anaerobic and facultative bacteria.⁽²⁾ Barnes et al used a solid (agar) version of the medium⁽²⁾ to develop vegetative cells in assays of *Clostridium perfringens* Clostridia. Reinforced Clostridial Medium was used in the enumeration of clostridia from food.⁽³⁾

Principles of the Procedure

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 litre of media.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Beef extract	10.00
Yeast extract	3.00
Dextrose	5.00
Sodium chloride	5.00
Sodium acetate	3.00
Starch, soluble	1.00
L-Cysteine hydrochloride	0.50
Agar	13.50
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing *C. botulinum* or *C. tetani* or their toxins.
4. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of these organisms and for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin.

Directions

1. Suspend 51 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and dispense as desired.





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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 5.1% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.35% Agar gel

Expected Cultural Response: Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35-37°C for 40-48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Bacteroides fragilis</i> ATCC 23745	50 -100	good-luxuriant	≥50%
2.	<i>Bacteroides vulgatus</i> ATCC 8482	50 -100	good-luxuriant	≥50%
3.	<i>Clostridium butyricum</i> ATCC 13732	50 -100	good-luxuriant	≥50%
4.	<i>Clostridium perfringens</i> ATCC 13124	50 -100	good-luxuriant	≥50%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Material to be examined is homogenized in a stomacher, and dilutions are prepared.
2. For enumeration, pour plate technique is employed. Incubate anaerobically.
3. If tubes are used, they are covered with sealing Anaerobic Agar immediately after the Reinforced Clostridium Medium has solidified.

Results

Refer appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Reinforced Clostridial Agar

Product Code : DM223

Available Pack sizes : 100gm / 500gm





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References

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.

Further Information

For further information please contact your local MICROMASTER Representative.



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