Technical Data Sheet

Reinforced Clostridial Agar (RCA)

Ordering number: 1.05410.0500

Medium proposed by BARNES and INGRAM (1956) for the cultivation and enumeration of clostridia, other anaerobes and facultative microorganisms in foodstuffs and other materials.

MUNOA and PARES (1988) developed a Bifidobacterium Iodoacetate Medium (BIM-25) on the basis of Clostridial Agar for the selective cultivation and differentiation of Bifidobacterium species.

Mode of Action

This culture medium is free from inhibitors and contains cysteine as a reducing agent. According to HIRSCH and GRINSTED (1954), Polymyxin B can be added to inhibit Gram-negative bacteria.

Typical Composition (g/L)

Reinforced Clostridial Agar	
Meat extract*	10.0
Peptone from casein	10.0
Yeast extract	3.0
D(+)glucose	5.0
Starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
L-cysteinium chloride	0.5
Agar-agar**	12.5

* Meat extract is equivalent to the term beef extract.
**Agar-agar is equivalent to other different terms of agar.

Preparation

Suspend 50 g/litre, if desired dispense into test tubes, autoclave (15 min at 121 °C). If required, cool to 45-50 °C and add 0.02 g Polymyxin B/litre in form of a filter-sterilized aqueous solution.

pH: 6.8 ± 0.2 at 25 °C.

The medium in the tubes or Petri dishes is clear and yellowish-brown.

Experimental Procedure and Evaluation

Prepare stab cultures of the sample material in test tubes or use the pour-plate technique.

Incubation: 24-48 hours at an optimal temperature (e.g. 35 °C) under anaerobic conditions (e.g. Anaerocult® A, Anaerocult® A mini, or Anaerocult® P).

Count the colonies and, if necessary, perform additional tests.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium.
Protect from UV light (including sun light). For *in vitro* use only.

Quality Control

Control strains	Growth after 48h at 35°C*
Clostridium bifermentans ATCC® 19299	good / very good
Clostridium novyi ATCC® 17861	good / very good
Clostridium sporogenes ATCC® 19404 (WDCM 00008)	good / very good
Clostridium perfringens ATCC® 13124 (WDCM 00007)	good / very good
Clostridium perfringens ATCC® 10543	good / very good
Escherichia coli ATCC® 25922 (WDCM 00013)	good / very good
Bacillus cereus ATCC® 11778 (WDCM 00001)	good / very good

*QC strains were mixed in tubes with liquefied RCA, then solidified to generate anaerobic conditions and placed in the incubator

Please refer to the actual batch related Certificate of Analysis.

Literature

BARNES, E.M., a. INGRAM, M.: The effect of redox potential on the grown Clostridium welchii strain isolated from horse muscle. – **J. Appl. Bact., 19**; 177-178 (1956).

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. – **J. Dairy Res., 21**;101-110 (1954).

MUNOA, F.J., a. PARES, R.: Selective medium for isolation and enumeration of Bifidobacterium spp. – **Appl. Environ. Microbiol., 54**; 1715-1718 (1988).

Ordering Information

Product	Cat. No.	Pack size
Reinforced Clostridial Agar (RCA)	1.05410.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Plate basket	1.07040.0001	1 ea
Polymyxin-B-sulfate	Calbiochem 5291-1GM	1 g

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