

CM 21496 – 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 Tryptone 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
Bacteroides fragilis	23745	50-100	Good-luxuriant	$\geq 50\%$	35-37°C	40-48 Hours
Bacteroides vulgatus	8482	50-100	Good-luxuriant	$\geq 50\%$	35-37°C	40-48 Hours
Clostridium butyricum	13732	50-100	Good-luxuriant	$\geq 50\%$	35-37°C	40-48 Hours
Clostridium perfringens	13124	50-100	Good-luxuriant	$\geq 50\%$	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

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