

CM 21497 – REINFORCED CLOSTRIDIAL AGAR (VEG.)

INTENDED USE

For cultivation and enumeration of Clostridia and other anaerobes.

PRODUCT SUMMARY AND EXPLANATION

Reinforced Clostridial Agar(Veg) is specially developed using Veg hydrolysate and Veg extract to avoid BSE/TSE risks associated with animal origin peptones and extracts. Reinforced Clostridial Agar (Veg) Media are the modifications of Reinforced Clostridial Media which are formulated by Hirsch and Grinstead. It can be used to initiate growth from small inocula and to obtain the highest viable count of Clostridia. This medium like the conventional medium can be used for diluting an inoculum of vegetative cells of Clostridium perfringens as suggested by Barnes and Ingram or can be used in studies of sporeforming anaerobes, especially Clostridium butyricum in cheese, also for the enumeration of Clostridia in tube dilution counts and for preparation of plates for isolation. Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media. These are enriched but nonselective media.

COMPOSITION

Ingredients	Gms / Ltr
Veg hydrolysate	10.000
Veg extract	10.000
Yeast extract	3.000
Dextrose	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 Veg hydrolysate, yeast extract, veg extract and starch, that provide all the necessary nutrients for the growth of Clostridia. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. Cystine hydrochloride is the reducing agent whereas sodium acetate acts as buffer. These media 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 10 psi pressure (115°C) for 15 minutes.

QUALITY CONTROL SPECIFICATIONS



Appearance of Powder	: Light yellow coloured may have slightly greenish tinge, 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	≥70%	35-37°C	40-48 Hours
Bacteroides vulgatus	8482	50-100	Good-luxuriant	≥70%	35-37°C	40-48 Hours
Clostridium butyricum	9690	50-100	Good-luxuriant	≥70%	35-37°C	40-48 Hours
Clostridium perfringens	13124	50-100	Good-luxuriant	≥70%	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. Hirsch and Grinstead, 1954, J. Dairy Res., 21:101.
2. Barnes and Ingram, 1956, J. Appl. Bact., 19:117.
3. Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

