

TM 2446 – CYCLOSERINE-CEFOXITIN FRUCTOSE AGAR (CCFA)

INTENDED USE

For the isolation and presumptive identification of *Clostridium difficile*, a recognized cause of pseudomembraneous (antimicrobial agent-associated) colitis.

PRODUCT SUMMARY AND EXPLANATION

Cycloserine-Cefoxitin Fructose Agar (CCFA) is an enriched, selective, and differential medium recommended for the isolation and presumptive identification of *Clostridium difficile*.

COMPOSITION

Ingredients	Gms / Ltr
Proteose peptone	40.000
Agar	15.000
Sodium phosphate Dibasic	5.000
Sodium chloride	2.000
Fructose	6.000
Potassium Phosphate Monobasic	1.000
Magnesium sulphate Heptahydrate	0.200
Neutral red	0.0003

PRINCIPLE

The medium consists of animal peptones and fructose as a source of nutritional compounds essential for the growth of bacteria. Cefoxitin and cycloserine are added in the medium for inhibiting the growth of most normal fecal flora. Cycloserine inhibits the gram-negative bacteria while cefoxitin inhibits both gram-positive and gram-negative organisms. Neutral red is added as a pH indicator which is turned yellow from pink/orange due to amino acids being utilized by the organism that increases the overall pH causing color change.

INSTRUCTION FOR USE

- Dissolve 69.23grams in 995ml distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes
- Cool to 45-50°C. Add rehydrated contents of one vial of Cycloserine –Cefoxitin Supplement, dissolved in
- 5ml Sterile distilled water.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder** : Beige colour, homogeneous free flowing powder.
Appearance of prepared medium : Pinkish-Orange, translucent gel.
pH (at 25°C) : 7.2 ± 0.2

INTERPRETATION



Cultural characteristics observed after incubation under anaerobic condition with added Cycloserine–Cefoxitin Supplement. Recovery rate is considered 100% for bacteria growth on Soya Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Appearance	Incubation temperature	Incubation Period
<i>Clostridium difficile</i>	9689	50-100	Luxuriant	>=70%	Yellow colored colonies, chartreuse fluorescence	35-37°C	18-48 Hours
<i>Bacteroides fragilis</i>	25285	>=10 ³	Inhibited	0%	-	35-37°C	18-48 Hours
<i>Escherichia coli</i>	25922	>=10 ³	Inhibited	0%	-	35-37°C	18-48 Hours
<i>Staphylococcus aureus</i>	25923	>=10 ³	Inhibited	0%	-	35-37°C	18-48 Hours

PACKAGING:

In pack size of 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.

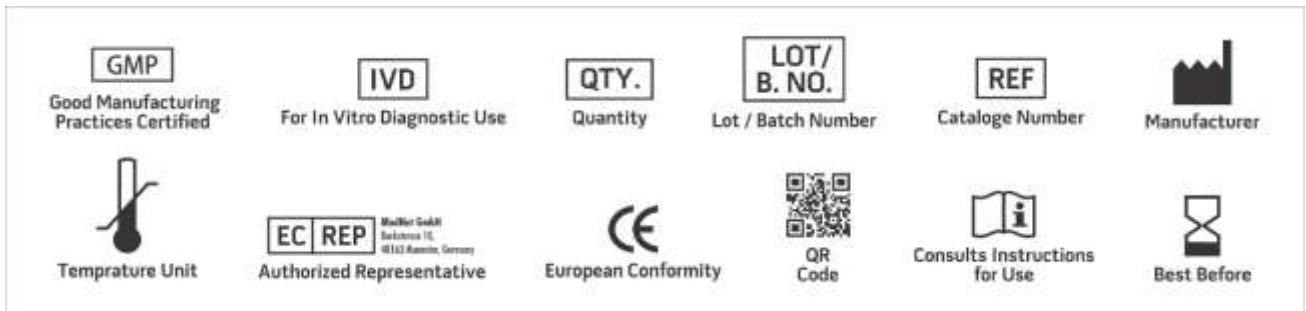
DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- Dowell, V. R., Jr. and T. M. Hawkins. 1987. Laboratory Methods in Anaerobic Bacteriology. CDC Laboratory Manual. USDHHS CDC. Atlanta, GA 30333.
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- Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson and A. Y. Armfield. 1977. Media for the Isolation, Characterization, and Identification of Obligately Anaerobic Bacteria. USDHHS, CDC, Atlanta, GA 30333.
- Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1987. Anaerobe Laboratory Manual. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061.
- Jousimies-Somer, H. R., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler and S. M. Finegold. 2002. Wadsworth –KTL Anaerobic Bacteriology Manual. Star Publishing Co., Belmont, CA 94002.





NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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