Anaerobe Systems
THE OXYGEN-FREE SPECIALISTS
A BIOLOG BRAND

INSTRUCTIONS FOR USE

CYCLOSERINE CEFOXITIN FRUCTOSE AGAR (CCFA)

PRODUCTS

AS-213 Cycloserine Cefoxitin Fructose Agar (CCFA) 1 plate / pkg

INTENDED PURPOSE

TruPRAS™ Anaerobic Culture Media is intended for the transport, preservation, or cultivation of a wide variety of microorganisms from specimens to aid in the isolation of bacteria for in vitro diagnostic and / or research / general laboratory purposes.

INTENDED USERS

Scientists, laboratory, and healthcare professionals trained in anaerobic microbiology techniques working in areas such as clinical, research, industrial, pharmaceutical and veterinary applications.

FORMULATION*

CCFA is an enriched, selective, and differential medium used for the growth of *Clostridioides difficile*, a recognized cause of pseudomembranous (antimicrobial-associated) colitis. The nutritive base consists of animal peptones and fructose, providing essential nutrients for *C. difficile*. The medium is supplemented with cycloserine and cefoxitin at concentrations that inhibit the growth of most normal fecal flora: cycloserine suppresses gram-negative bacteria, while cefoxitin inhibits both gram-positive and gram-negative organisms. *Clostridioides difficile* is not inhibited and typically produces distinctive yellow, ground-glass colonies on CCFA. Neutral red is included as a pH indicator. As *C. difficile* metabolizes amino acids, the resulting alkaline byproducts increase the pH, shifting the indicator from pink/orange to yellow around the colonies. This medium is prepared, dispensed, and packaged under oxygen-free conditions using TruPRAS™ Technology to prevent the formation of oxidized products prior to use. This product is supplied ready to use, with no pre-reduction step required.

FORMULATION*

Proteose peptone	40.00	g
Sodium phosphate dibasic	5.00	g
Potassium phosphate monobasic	1.00	g
Sodium chloride	2.00	g
Magnesium sulfate heptahydrate	0.20	g
D-Fructose	6.00	g
Agar	15.00	g
Neutral red	0.03	g
Cycloserine	0.50	g
Cefoxitin	15.60	mg
DI Water	1.00	L

*Approximate formula. Adjusted and/or supplemented as required to meet performance criteria.

Final pH: 7.2 ± 0.2 at 25°C
Final weight: 16.0 g ± 1.6 g

PRECAUTIONS

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use by properly trained and qualified personnel only. Sterilize all biohazard waste prior to disposal. This product is manufactured as a single use device.

Report serious incidents that occur in direct relation to this product to tech@biolog.com. As necessary, report serious incidents to the regulatory authority in which the user is established.

This product may contain components of animal origin. All components of animal origin have been sourced from Bovine Spongiform Encephalopathy- (BSE-) free and Transmissible Spongiform Encephalopathy- (TSE-) free countries. Certified knowledge of the origin of animal derived components does not guarantee the absence of transmissible pathogenic agents. It is recommended that Universal Precautions be observed.

When working with anaerobic culture media, the potential for ergonomic hazards may exist due to repetitive motions, awkward postures, improper bench/chair heights or poor lighting. Although it is beyond the scope and provision of products by Anaerobe Systems, it should be recognized and mitigated by the end user in the laboratory environment.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2 – 8°C in original package until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 90 days from the date of manufacture.

PROCEDURE

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes. The selection of specimens for culture is made by physicians or scientists collecting the sample.

Methods for Use: Remove CCFA from 2 – 8°C storage and allow it to reach room temperature before use. CCFA may be inoculated directly with a specimen or from a broth previously inoculated with a specimen. Streak the plate with the inoculum to obtain isolated colonies. Immediately place the plate in an anaerobic atmosphere and incubate at 35 – 37°C for 18 to 48 hours. Quantification of *Clostridioides difficile* in a specimen may provide clinically useful information. This can be performed by thoroughly mixing a serial 10-fold dilution of the specimen under anaerobic conditions and plating the dilutions onto CCFA. For detailed procedures on anaerobic culture handling and processing, refer to the appropriate references.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

INTERPRETATION OF RESULTS

CCFA supports robust growth of *Clostridioides difficile*. After 24 to 48 hours of incubation, most C. difficile colonies appear large, circular, and yellow. The yellow coloration typically extends 2 – 3 mm beyond the colony margin into the surrounding pink/orange medium. When exposed to long-wavelength ultraviolet (UV) light, these colonies exhibit a characteristic golden-yellow to chartreuse fluorescence.

LIMITATIONS

CCFA does not provide complete identification for the definitive identification of bacterial isolates. Additional testing is necessary. Rare strains of *Clostridioides difficile* may be inhibited by the selective agents present in the medium. Plates should be examined within 48 hours of incubation to ensure optimal selectivity. If incubation is extended to 3 to 5 days, significant growth of non-C. difficile organisms may occur. These colonies are often small (pinpoint to 0.5 mm in diameter) and typically do not exhibit the characteristic golden-yellow or chartreuse fluorescence under long-wavelength UV light. To confirm the obligate anaerobic nature of each colony type, a test for aerotolerance should be performed. For further information on identification and confirmation, consult appropriate reference materials.

QUALITY CONTROL

The following organisms are routinely used for quality control testing at Anaerobe Systems using the specifications outlined in the CLSI document M22-A3: Quality Control for Commercially Prepared Microbiological Culture Media.

Organism Tested	ATCC® #	Results	Special Reactions
Bacteroides fragilis	25285	No growth	
Prevotella melaninogenica	25845	No growth	
Fusobacterium necrophorum	25286	No growth	
Fusobacterium nucleatum	25586	No growth	
Clostridium perfringens	13124	No growth	
Peptostreptococcus anaerobius	27337	No growth	
Staphylococcus aureus	25923	No growth	
Enterococcus faecalis	29212	No growth	
Escherichia coli	25922	No growth	
Clostridioides difficile	9689	Growth	Yellow coloration & chartreuse fluorescence

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If the nutritive/inhibitory capacity of this medium is to be tested for performance, it is recommended that the following ATCC® organisms be evaluated for growth.

Organism	ATCC® #	Results	Special Reactions
Bacteroides fragilis	25285	No growth	
Escherichia coli	25922	No growth	
Staphylococcus aureus	25923	No growth	
Clostridioides difficile	9689	Growth	Yellow coloration & chartreuse fluorescence

Physical Appearance: CCFA should appear translucent pink/orange in color.

ATCC® is a registered trademark of American Type Culture Collection.

REFERENCES

1. CLSI. *Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline*. CLSI document M56-A. Clinical and Laboratory Standards Institute; 2014

2. Leber AL, Burnham CA, eds. *Clinical Microbiology Procedures Handbook*. 5th ed. 4 vols. Washington, DC: ASM Press; 2023.

3. Carroll KC, Pfaller MA, eds. *Manual of Clinical Microbiology*. 13th ed. 4 vols. Hoboken, NJ: Wiley-Blackwell; 2023.

4. Jousimies-Somer HR, Sutter VL, eds. *Wadsworth-KTL Anaerobic Bacteriology Manual*. 6th ed. Belmont, CA: Star Publishing Company; 2002.

5. CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition.* CLSI document M22-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.

6. George WL, Sutter VL, Citron D, Finegold SM. *Selective and differential medium for isolation of Clostridium difficile*. J Clin Microbiol. 1979;9(2):214-219.

7. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. *Animal Health Status of Regions*. Published March 12, 2025. https://www.aphis.usda.gov/regionalization-evaluation-services/region-health-status

8. European Commission. *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 Rev. 3)*. Published March 5, 2011. https://op.europa.eu/en/publication-detail/-/publication/3392e464-ba89-4ae4-955c-a07f617c8e06/language-en

GLOSSARY OF SYMBOLS

SYMBOL	TITLE	DESCRIPTION	STANDARD	REF#
	Catalog number	Indicates the manufacturer's catalog number so that the medical device can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.1
	Lot number/ Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labeling, and information to be supplied	5.1.5
	Use-by date	Indicates the date after which the medical device is not to be used.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.4
	Authorized Representative	Indicates the Authorized Representative in the identified country or jurisdiction.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.2
	Do not re-use/ Single use only	Indicates a medical device that is intended for one single use only.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.2
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.3
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.3.7
	In vitro diagnostic medical device	Indicates that a medical device is intended to be used as an in vitro diagnostic medical device	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.5.1
	CE Mark European Conformity	Designates that the product labeled Is authorized for sale in European countries.	EU IVDR (EU) 2017/746	

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AUTHORIZED REPRESENTATIVE INFORMATION

EC REP
Casus Europe B.V.
Lange Viestraat 2b
3511 BK Utrecht
The Netherlands

CH REP
Casus Switzerland GmbH
Hinterbergstrasse 49
6312 Steinhausen
Switzerland

REVISION 4
Additions: Intended Use, Intended Users, Animal Origin Statement, Ergonomics Precautions, Serious Incident Report Contact Information, Glossary of Symbols
Changes: Title change from Product Insert to Instructions for Use. References updated. Contact information.
Deletions: None