

## CLOSTRIDIUM DIFFICILE AGAR (7385)

### Intended Use

**Clostridium Difficile Agar** is used with blood, Cycloserine, and Cefoxitin for the isolation of *Clostridium difficile* in a laboratory setting. Clostridium Difficile Agar is not intended for use in the diagnosis of disease or other conditions in humans.

### Product Summary and Explanation

*Clostridium difficile* has been isolated from diverse natural habitats, including soil, hay, sand, dung from various large mammals, and from dog, cat, and rodent feces.<sup>1</sup>

In 1979, George et al. developed a medium called CCFA (Cycloserine-Cefoxitin-Fructose agar), based on the Egg Yolk Agar formula of McClung and Toabe with fructose replacing glucose.<sup>2</sup> Clostridium Difficile Agar is a modification of the original CCFA formulation.

### Principles of the Procedure

Proteose Peptone provides nitrogen, vitamins, and amino acids in Clostridium Difficile Agar. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile*. The Phosphates are buffering agents in this medium. Magnesium Sulfate is a source of inorganic ions to stimulate growth. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Horse blood provides essential growth factors in Clostridium Difficile Agar. Cycloserine and Cefoxitin are selective agents against aerobic, anaerobic, and facultatively anaerobic Gram-positive and Gram-negative bacteria. At the concentration of antibiotics used *C. difficile* is not inhibited significantly, while other anaerobes, including most clostridia, are inhibited.

### Formula / Liter

Enzymatic Digest of Casein .....	15 g
Enzymatic Digest of Animal Tissue .....	15 g
Pork Brain Heart Infusion Solids.....	10 g
Fructose .....	6 g
Disodium Phosphate.....	5 g
Monopotassium Phosphate .....	1 g
Magnesium Sulfate .....	0.1 g
Sodium Chloride .....	2 g
Agar .....	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Supplements

Horse Blood, 7%  
Cycloserine, 0.5 g/L  
Cefoxitin, 0.016 g/L

### Precautions

1. For Laboratory Use Only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 69 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 7% horse blood, Cycloserine (0.5 g/L) and Cefoxitin (0.016 g/L).

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and light beige.

**Prepared Appearance:** Prepared medium with supplements is opaque and red.

**Expected Cultural Response:** Cultural response on Clostridium Difficile Agar supplemented with 7% horse blood, Cycloserine, and Cefoxitin and incubated at 35 ± 2°C under the appropriate atmosphere and examined for growth after 24 - 72 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
		Growth
<i>Bacteroides fragilis</i> ATCC® 25285	10 <sup>3</sup>	Inhibited
<i>Clostridium difficile</i> ATCC® 17858	10 - 300	Growth
<i>Clostridium perfringens</i> ATCC® 13124	10 <sup>3</sup>	Inhibited
<i>Clostridium sporogenes</i> ATCC® 11437	10 <sup>3</sup>	Inhibited
<i>Staphylococcus aureus</i> ATCC® 25923	10 <sup>3</sup>	Inhibited

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

For a complete discussion on the isolation and identification of *C. difficile* and other anaerobic bacteria refer to specific procedures in appropriate references.<sup>1,3</sup>

### Results

Colonies of *C. difficile* are 4 - 6 mm in diameter, irregular, raised, opaque, and grey-white after 48 hours incubation.

### Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *C. difficile*.
2. Clostridium Difficile Agar does not contain Neutral Red indicator because it is designed for use with horse blood.<sup>2</sup>
3. Typical Gram stain morphology of *C. difficile* may not be evident in colonies picked from this medium because of antibiotics present. Subculture suspected colonies to blood agar to obtain characteristic morphology.

### Packaging

<b>Clostridium Difficile Agar</b>	<b>Code No.</b>	<b>7385A</b>	<b>500 g</b>
		<b>7385B</b>	<b>2 kg</b>
		<b>7385C</b>	<b>10 kg</b>

### References

1. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
2. George, W. L., V. L. Sutter, D. Citron, and S. M. Finegold. 1979. Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. 9:214.
3. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.



620 Leshar Place, Lansing MI 48912  
517/372-9200 • 800/783-3212 • fax: 800/875-8563  
neogen-info@neogen.com • www.neogen.com