

## Clostridium Difficile Agar, CCFA (i23075)

Cycloserine Cefoxitin Fructose Agar Base (CCFA) is used with blood, Cycloserine, and Cefoxitin for the isolation of *Clostridium difficile*.

Industry: Food / Clinical

## Principles & Uses

Clostridium Difficile Agar Base, designed for isolating *Clostridium difficile* from fecal specimens, uses selective agents like D-cycloserine and cefoxitin to inhibit the growth of various bacteria present in large quantities in feces. The medium, initially proposed by George et al., incorporates fructose, peptone, and specific buffering agents to facilitate the growth of *C. difficile*. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile*. Potassium dihydrogen phosphate and disodium hydrogen phosphate act as a buffer system. Magnesium sulfate is an ion required in a large variation of enzymatic reactions, including DNA replication. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Agar is the solidifying agent. This agar base, used with horse blood, enhances recovery and increases colony size. The presence of antibiotics in the medium influences the typical gram stain morphology of *C. difficile* colonies, necessitating subculture on Blood Agar for characteristic morphology. The spectrum of diseases caused by *C. difficile* includes conditions like pseudomembranous colitis and antibiotic-associated colitis.

The isolation process involves spreading fecal samples on the medium, followed by anaerobic incubation, resulting in distinct colonies of *C. difficile* with specific characteristics. However, for comprehensive identification and confirmation, additional tests like gram staining, morphology, biochemical assays, specific cytotoxin tests, and clinical observations are essential.

## Composition (gr/L)

Proteose Peptone 40 g, Fructose 6 g, Disodium hydrogen Phosphate 5 g, Sodium Chloride 2 g, Potassium dihydrogen phosphate 1 g, Magnesium sulfate heptahydrate 0.1 g, Agar 15 g.

Final pH at 25°C 7.4 ± 0.2

## Preparation from dehydrated Powder

Suspend 69.1 g of the medium in 1 liter of purified water. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 7% horse blood, and contents of 2 vials of CCFA Supplement.

## Quality Control

Dehydrated Appearance: Cream to yellow, free-flowing, homogeneous.

Prepared Appearance: Basal medium: Light amber, clear to slightly opalescent gel.

With 7% v/v defibrinated horse blood: Cherry red, opaque.

Reaction of 6.91% Solution at 25°C: pH 7.4 ± 0.2

## Cultural Response

Cultural response was observed under anaerobic condition after incubation at 35 - 37 °C for 48 hours.

| Organism (ATCC*)                     | Recovery  | Colony color  |
|--------------------------------------|-----------|---------------|
| <i>Clostridium difficile</i> (11204) | good      | greyish-white |
| <i>Shigella flexneri</i> (12022)     | inhibited | -             |
| <i>Escherichia coli</i> (25922)      | inhibited | -             |
| <i>Staphylococcus aureus</i> (25923) | inhibited | -             |

\*ATCC is a registered trade mark of the American Type Culture Collection.

## Storage

Keep the container at 15-30 °C. Store prepared medium at 2-8 °C.