

Selective medium for the isolation of *Clostridioides difficile* from feces.

Formula in g/L			
Proteose peptone.....	40,00	Fructose.....	6,00
Sodium chloride.....	2,00	Magnesium sulphate.....	0,10
Disodium hydrogen phosphate.....	5,00	Potassium dihydrogen phosphate.....	0,90
Agar.....	15,00		

Final pH at 25°C: 7,3 ± 0,2

Principle:

Clostridium difficile LAB-AGAR™ is a selective medium for the isolation of *Clostridioides difficile*. Proteose peptone provides nitrogen, vitamins, mineral and amino acids essential for growth. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile*. Disodium phosphate and monopotassium phosphate act as a buffer system. Magnesium sulphate ion required in a large variation of enzymatic reactions, including DNA replication. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sheep blood provides essential growth factors in *Clostridium difficile* LAB-AGAR™. The selective agents D-Cycloserine and cefoxitin inhibit the growth of most Enterobacteriaceae, as well as *E. faecalis*, staphylococci, non-sporing anaerobic bacilli and *Clostridium* spp. (except *C. difficile*), which may be found in large quantities in fecal samples.

Preparation: suspend 34.5 grams of the medium in 460 ml of distilled water. Mix well and dissolve by heating with frequent agitation until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C and 35 ml defibrinated sheep blood and one vial of *Clostridium Difficile* Selective Supplement (ref. SL 0048), previously reconstituted in 4 ml of sterile distilled water and 7% sterile defibrinated horse blood. Homogenize gently and dispense into Petri dishes. Be careful to avoid bubble formation when adding the blood and rotate the flask or bottle slowly to create a homogeneous solution.

The prepared medium should be stored at 8-15°C.

Procedure:

- ★ Use the medium with the normal laboratory procedures, incubate the inoculated plates at 37°C for 24-48 hours in anaerobic conditions

Result:

- ★ After 48 hours *Clostridium difficile* colonies grow circular, raised, opaque grey, sometimes with irregular borders, and 4-6 mm in diameter.

Storage / Shelf life

- ★ Once opened keep powdered medium closed to avoid hydration at 2 - 30°C
- ★ The expiration date is indicated on the label.

Packaging: 500 g

Supplement: *Clostridium difficile* Selective Supplement 10 vials. 1 vial /500 ml

Ref. SL 0048

