

## CLOSTRIDIUM DIFFICILE AGAR BASE

Medium for *Clostridium difficile* isolation from feces.

TYPICAL FORMULA	(g/l)
Peptospecial	40.0
Sodium Phosphate Dibasic	5.0
Potassium Phosphate Monobasic	1.0
Magnesium Sulphate	0.1
Sodium Chloride	2.0
Fructose	6.0
Agar	15.0

Final pH = 7.4 ± 0.2 at 25 °C.

### CLOSTRIDIUM DIFFICILE SUPPLEMENT

1 vial content (1 vial is sufficient for 500 ml of medium):

D-Cycloserine	125.0 mg	(equivalent to 250 mg/l of the medium)
Cefoxitin	4.0 mg	(equivalent to 8 mg/l of the medium)

### DIRECTIONS

Suspend 34.6 g of powder in 500 ml of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and add aseptically 1 vials of Clostridium difficile supplement (D-cycloserine 125 mg, cefoxitin 4 mg) (code 81007) and 25 ml of defibrinated sheep blood (code 83296). Final medium will contain D-cycloserine 250 mg/l and cefoxitin 8 mg/l. Mix well. Dispense in petri dishes.

### DESCRIPTION

CLOSTRIDIUM DIFFICILE AGAR, added with Clostridium difficile supplement, is a selective medium for *Clostridium difficile* isolation from feces. Cycloserine and cefoxitin inhibit the growth of a large variety of microorganisms, included enterobacteria, fecal streptococci, anaerobic non-spore forming gram-negative bacilli and *Clostridium*, except *C. difficile*. *C. difficile* grows after 48 hours of incubation at 37 °C in anaerobic conditions, with 4-6 mm diameter, irregular, raised, opaque, grey-white colonies.

### TECHNIQUE

#### Direct faces inoculation

Lightly inoculate the medium with the fecal sample by spreading part of the initial suspension in order to obtain isolated colonies and incubate at 36 ± 1 °C for 24-48 hours in anaerobic atmosphere.

#### Alcohol shock treatment

Mix equal parts of absolute or 95% ethylic alcohol and the fecal suspension. Homogenize using a vortex mixer. Leave at room temperature for 1 hour. Inoculate onto Clostridium Difficile Agar and incubate at 36 ± 1 °C for 24-48 hours anaerobically.

### QUALITY CONTROL

#### Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: beige.

#### Prepared medium

Appearance: clear.

Color: light amber.

Incubation conditions: 36 ± 1 °C for 24-48 hours, anaerobically.

Microorganism	ATCC	Growth
<i>Escherichia coli</i>	25922	none
<i>Clostridium difficile</i>	11204	good

### STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.

Store prepared plates at 2-8 °C.



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## REFERENCES

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2. Barlett, J.G. et al. (1978) N. Eng. J. Med., **298**, 531.
3. Boriello, S.P. et al (1981) J. Antimicrob. Chemother. 7 Supp. A. 53-62.
4. George, R.H. et al (1976) J. Clin. Microbiol. **6**, 214-219.

## PRESENTATION

Product	REF	$\Sigma$
CLOSTRIDIUM DIFFICILE AGAR BASE (7.1 l)	610115	500 g
CLOSTRIDIUM DIFFICILE AGAR BASE (1.4 l)	620115	100 g
CLOSTRIDIUM difficile supplement	81007	10 vials
SHEEP BLOOD DEFIBRINATED	83296	50 ml

## TABLE OF SYMBOLS

<b>LOT</b> Batch code	 Caution, consult accompanying documents	 Manufacturer	 Contains sufficient for <n> tests	<b>IVD</b> <i>In Vitro</i> Diagnostic Medical Device
<b>REF</b> Catalogue number	 Fragile, handle with care	 Use by	 Temperature limitation	 Keep away from heat source



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