

Materials List for:

Culturing and Maintaining *Clostridium difficile* in an Anaerobic Environment

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Materials

Name	Company	Catalog Number	Comments
Proteose Peptone no. 2	BD	212120	
Na ₂ HPO ₄	Fisher	S373	
KH ₂ PO ₄	Fisher	BP362	
NaCl	Fisher	S27	
MgSO ₄ (anhydrous)	Fisher	M65	
α-D-Fructose	Fisher	L96	
Sodium taurocholate	Sigma	T4009	
α-D-cycloserine	Sigma	C6880	
Cefoxitin	Fluka	C4786	
Brain heart infusion medium	BD	237300	
Proteose Peptone	BD	211684	
(NH ₄) ₂ SO ₄	Sigma	A5132	
Tris base	Fisher	BP152	
Agar	BD	214010	
L-cysteine	Sigma	C7755	
BactoPeptone	BD	211684	
Columbian sheep blood agar	Fisher	L21928	
NaCl	Fisher	S27	
KCl	Fisher	P217	
Glycerol	Fisher	BP2291	
Sterile inoculating loops	Fisher	22363596	
Sterile swabs	Fisher	1495990	
Coy Vinyl Anaerobic Chamber and Accessories	Coy Laboratory Products, Inc	Customer Specified	These items are custom ordered per laboratory needs

Materials

TCCFA agar

Proteose peptone no. 2 (Difco) 40 g

Na₂HPO₄ 5 g

KH₂PO₄ 1 g

NaCl 2 g

MgSO₄ (anhydrous) 0.1 g

Fructose 6 g

Agar 20 g

Bring to 1 L with deionized water and autoclave at 121 °C for 20 min to sterilize.

After autoclaving, add:

10 ml of 10% (w/v) sodium taurocholate, filter-sterilized (dissolve in water; final concentration, 0.1%)

25 ml of 10 mg/ml α-D-cycloserine, filter-sterilized (dissolve in water; final concentration, 250 µg/ml)

1.6 ml of 10 mg/ml cefoxitin, filter-sterilized (dissolve in water; final concentration, 16 µg/ml)

BHIS Medium

Brain heart infusion 37 g

Yeast extract 5 g

For plates, add 15 g agar. Bring to 1 L with deionized water and autoclave at 121 °C for 20 min to sterilize.

Optional (add after autoclaving):

3 ml of 10% (w/v) L-cysteine (dissolve in water; final concentration, 0.03%)

10 ml of 10% (w/v) sodium taurocholate (dissolve in water; final concentration, 0.1%)

SMC Sporulation Medium

BactoPeptone 90 g

Protease peptone 5 g

(NH₄)₂SO₄ 1 g

Tris base 1.5 g

Agar 15 g

Bring to 1 L with deionized water and autoclave at 121 °C for 20 min to sterilize.

Optional (add after autoclaving):

3 ml of 10% (w/v) L-cysteine (dissolve in water; final concentration, 0.03%)

70:30 Medium

BactoPeptone 63 g

Protease peptone 3.5 g

Brain heart infusion 11.1 g

Yeast extract 1.5 g

(NH₄)₂SO₄ 0.7 g

Tris base 1.06 g

For plates, add 15 g agar. Bring to 1 L with deionized water and autoclave at 121 °C for 20 min to sterilize. After autoclaving, add 3 ml of 10% (w/v) L-cysteine (final concentration, 0.03%).

Blood agar

The use of premade Columbia anaerobic sheep blood agar plates (Fisher Scientific, L21928)³⁵ is recommended.

1X Phosphate buffered saline (PBS)

NaCl 8.01 g

KCl 0.2 g

Na₂HPO₄ 1.44 g

KH₂PO₄ 0.27 g

Bring to 1 L with deionized water and adjust pH to 7.4 with HCl. Filter sterilize before use.