

Buffered Charcoal Yeast Extract (BCYE) Agar, pH 6.9 – 1 L

Introduction

Buffered Charcoal Yeast Extract (BCYE) agar is used for the growth of the pathogenic gram-negative bacteria *Legionella* spp. It will appear as a black-grey colored gel. Yeast extract provides essential macronutrients for the bacteria. Activated charcoal breaks down hydrogen peroxide, a toxic metabolic product, and also modifies the surface tension of the medium. ACES buffer helps to maintain the optimal pH for growth. Alpha-Ketoglutarate stimulates growth and increases the sensitivity of the medium. Ferric pyrophosphate provides a source of iron. L-Cysteine is an amino acid that is essential for the growth of *Legionella* spp. Selective agents, such as polymyxin or vancomycin, can be added to inhibit the growth of other organisms. Dyes such as bromocresol purple and bromothymol blue can be added to help identify and differentiate between *Legionella* species. Glycine can be added to increase growth of *Legionella* spp. Bovine Serum Albumin is sometimes added to the medium if there is evidence of *Legionella* species other than *Legionella pneumophila* present.

Materials

- Yeast Extract
- ACES Buffer ([ACES, GoldBio Catalog # A-010](#) [CAS 7365-82-4, mw. = 182.20])
- Activated Charcoal
- Ferric pyrophosphate
- α -Ketoglutarate, Monopotassium Salt
- 4% (w/v) L-Cysteine HCl
- Agar
- pure water

Method

1. Weigh 10 g yeast extract.
2. Weigh 0.25 g ferric pyrophosphate.
3. Weigh 1.0 g α -ketoglutarate, monopotassium salt.
4. Weigh 1.5 g activated charcoal.
5. Weigh 15 g agar. Combine all ingredients into an autoclavable bottle.

6. Add 550 ml 0.1M ACES buffer, pH~6.9 (See [ACES Stock Solution](#) Protocol).
7. Fill to volume of 990 ml with dH₂O.
8. Sterilize with autoclave at 121°C for 15 minutes. Cool to 45-50°C.
9. Slowly add 10 ml sterile solution of 4% L-Cysteine.
10. If necessary, adjust pH with 1M KOH or 1M HCl.
 - a. Optional: Add sterile selective inhibitor agents if desired.
 - b. Optional: Add sterile 1.0% BSA solution if desired.
11. Pour media into petri plates and let stand until solidified.

Note: Medium should be mixed continuously until poured to keep charcoal particles suspended.

Calculations

A 4% solution of L-cysteine is made by suspending 0.4 g of L-cysteine in water and filling to a final volume of 10 ml.

Tips

- Every batch of BCYE agar medium should be tested for quality control. The pH should be checked and the ability to support growth should be checked.
- A fresh solution of L-cysteine should be prepared each time.

Associated Products

- Anisomycin ([Anisomycin, GoldBio Catalog # A-580](#) [CAS 22862-76-6, mw. = 265.30])
- Bovine Serum Albumin ([BSA, GoldBio Catalog # A-420](#) [CAS 9048-46-8])
- Bromothymol Blue ([Bromothymol Blue, GoldBio Catalog # B-750](#) [CAS 34722-90-2, mw. = 646.36])
- Bromocresol Purple
- Cycloheximide ([Cycloheximide, GoldBio Catalog # C-930](#) [CAS 66-81-9, mw. = 281.353])
- Fluconazole ([Fluconazole, GoldBio Catalog # F-450](#) [CAS 86386-73-4, mw. = 306.27])
- Glycine ([Glycine, USP Grade, GoldBio Catalog # G-630](#) [CAS 56-40-6, mw. = 75.07])
- Natamycin ([Natamycin, GoldBio Catalog # N-600](#) [CAS 7681-93-8, mw. = 665.73])
- Polymyxin B Sulfate ([Polymyxin B Sulphate, GoldBio Catalog # P-740](#) [CAS 1405-20-5, mw. = 1385.61])

- Vancomycin Hydrochloride ([Vancomycin, GoldBio Catalog # V-200](#) [CAS 1404-73-9, mw. = 1485.71])

References

Centers for Disease Control and Prevention (CDC). "Procedures for the Recovery of Legionella from the Environment." *National Center for Infectious Diseases. Division of Bacterial and Mycotic Diseases. Respiratory Disease Laboratory Section. US Department of Health and Human Services* (2005).

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