

BUFFERED CYE SELECTIVE AGAR (BCYE w/ GPCV)

INTENDED USE

Remel Buffered CYE Selective Agar w/ GPCV is a solid medium recommended for use in qualitative procedures for selective isolation of *Legionella pneumophila* from environmental samples.

SUMMARY AND EXPLANATION

In 1977, McDade et al. isolated the Legionnaires' disease bacterium using guinea pigs and embryonated chicken eggs.¹ In 1978, Feeley et al. developed a medium containing ferric pyrophosphate and L-cysteine hydrochloride for isolation of *Legionella* from clinical specimens.² In a later modification, Feeley substituted yeast extract and charcoal for casein hydrolysate and beef extract to create Charcoal Yeast Extract (CYE) Agar.³ Pasculle et al. modified CYE Agar by adding ACES buffer (N-2-acetamido-2-aminoethane-sulfonic acid) to create Buffered Charcoal Yeast Extract (BCYE) Agar.⁴ In 1981, Edelstein added α -ketoglutarate to BCYE Agar which improved the recovery of *Legionella pneumophila* from contaminated environmental samples.⁵ Dennis et al. recommended the use of BCYE w/ GPCV (i.e., glycine, polymyxin B, cycloheximide, and vancomycin) to recover *Legionella* spp. from environmental samples which are heavily contaminated with competing flora.⁶ BCYE w/ GPCV is also recommended by Centers for Disease Control and Prevention (CDC) for testing water samples.⁷

PRINCIPLE

BCYE w/ GPCV contains charcoal and yeast extract to enhance the growth of *Legionella*. Charcoal also serves to absorb toxic metabolic products and modify the surface tension of the medium. Ferric pyrophosphate and L-cysteine hydrochloride are added to satisfy the specific nutritional requirements of *Legionella*. ACES Buffer serves to maintain proper pH and α -ketoglutarate is added to stimulate growth. Polymyxin B, cycloheximide, and vancomycin are selective agents added to inhibit the growth of contaminating bacteria and yeasts. Glycine is a selective agent inhibitory to many bacteria found in environmental samples. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

ACES Buffer.....	10.0 g	Ferric Pyrophosphate.....	0.25 g
Yeast Extract.....	10.0 g	Cycloheximide.....	80.0 mg
Glycine.....	3.0 g	Vancomycin.....	1.0 mg
Charcoal.....	1.5 g	Polymyxin B.....	80,000 U
α -ketoglutarate.....	1.0 g	Agar.....	15.0 g
L-Cysteine Hydrochloride.....	0.4 g	Demineralized Water.....	1000.0 ml

pH 6.9 \pm 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

PROCEDURE

1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, testing, and interpretation.

QUALITY CONTROL

Each lot number of BCYE w/ GPCV has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

CONTROL

Legionella pneumophila ATCC® 33152
**Tatlockia micdadei* ATCC® 33204
Cryptococcus neoformans ATCC® 34877
Escherichia coli ATCC® 25922
Staphylococcus epidermidis ATCC® 12228

* Also referred to as *Legionella micdadei*

INCUBATION

Ambient, up to 72 h @ 33-37°C
Ambient, up to 72 h @ 33-37°C
Ambient, up to 72 h @ 25-30°C
Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C

RESULTS

Growth
Growth
No growth
No growth
No growth

LIMITATIONS

1. Gram-negative bacilli other than *Legionella* may grow on BCYE w/ GPCV Agar. Additional biochemical and/or serological tests are required for definitive identification of *Legionella* spp. Follow established laboratory procedures and consult appropriate references for further instructions.⁷
2. The selective agents contained in BCYE w/ GPCV may inhibit the growth of some *Legionella* spp. For optimum recovery of *Legionella*, use a nonselective BCYE Agar in parallel with BCYE w/ GPCV.⁸

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, sample collection, storage and transportation, materials required, quality control, and limitations.

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IFU 110101, Revised July 26, 2011

Printed in U.S.A.

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