

BD BBL™ Prepared Tubed Medium for Cultivation of Microorganisms D/E Neutralizing Broth

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INTENDED USE

D/E Neutralizing Broth is for the neutralizing and testing of antiseptics and disinfectants according to the procedure of Engley and Dey.¹

SUMMARY AND EXPLANATION

D/E Neutralizing Broth is used for environmental sampling where neutralization of the chemical is important to determine its bactericidal or bacteriostatic activity. This medium will neutralize a broad spectrum of antiseptic and disinfectant chemicals, including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and gluteraldehyde.¹

PRINCIPLES OF THE PROCEDURE

Casein peptone, yeast extract and dextrose are sources of nutrients required for the replication of microorganisms. Sodium thioglycollate neutralizes mercurials, sodium bisulfite neutralizes formaldehyde and gluteraldehyde and the sodium thiosulfate is able to neutralize iodine and chlorine. Lecithin neutralizes cationic surface-active agents, such as quaternary ammonium compounds; polysorbate 80, a non-ionic surface active agent, neutralizes phenolics.

Since the high concentration of lecithin renders the medium opaque, turbidity cannot be used to detect growth. Therefore, bromcresol purple and dextrose are added to the medium. Those organisms which ferment dextrose will turn the medium from purple to yellow. Growth of *Pseudomonas* species, which do not ferment dextrose, can be detected by the formation of a pellicle on the surface of the broth.¹

REAGENTS

Formula:

D/E Neutralizing Broth

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	5.0 g
Yeast Extract	2.5 g
Dextrose	10.0 g
Sodium Bisulfite	2.5 g
Sodium Thioglycollate	1.0 g
Sodium Thiosulfate	6.0 g
Lecithin	7.0 g
Polysorbate 80	5.0 g
Bromcresol Purple	0.02 g
Dipotassium Phosphate	3.3 g
Monopotassium Phosphate	0.1 g

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For Laboratory Use

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving.

Storage Instructions: On receipt, store tubes at 2 – 8°C. Freezing and overheating must be avoided. Allow the medium to warm to room temperature before inoculation. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

PROCEDURE

Material Provided: D/E Neutralizing Broth

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Add 1 mL of disinfectant solution to one tube of D/E Neutralizing Broth. Add culture as desired. Incubate tubes at 35°C. Examine for growth, indicated by a color change from purple to yellow or by pellicle formation.

To determine whether viable organisms are present in a bacteriostatic or bactericidal solution, inoculate samples from the broth onto D/E Neutralizing Agar or Standard Methods Agar plates. Incubate plates at 35 – 37°C for 48 h.

User Quality Control:

1. Examine tubes for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of tubes with pure cultures of stable control organisms that produce known, desired reactions. The following cultures are recommended:

TEST STRAIN

Staphylococcus aureus ATCC™ 25923

Pseudomonas aeruginosa ATCC 10145

EXPECTED RESULTS

Growth, yellow color

Growth, indicated by pellicle formation; no color change

RESULTS

If the disinfectant solution is bacteriostatic, it should be neutralized and the test organisms introduced into the broth will grow. Growth is indicated by a color change of the medium from purple to yellow, or pellicle formation.

Growth on the plates from negative broth tubes indicates a bacteriostatic substance. No growth on the plates from negative broth tubes indicates a bactericidal substance.

All positive broth tubes should be positive on the plates.

LIMITATIONS OF THE PROCEDURE

Appropriate references should be consulted for further information.^{1,2}

AVAILABILITY

Cat. No. Description

298318 BBL™ D/E Neutralizing Broth, Ctn. of 100 size A tubes

REFERENCES

1. Engley, F.B., Jr., and B.P. Dey. 1970. A universal neutralizing medium for antimicrobial chemicals. Chem. Spec. Manuf. Assoc. Proc., Mid-Year Meet., p. 100-106.
2. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

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