Northeast Laboratory Services

P.O. Box 788
Waterville, Maine 04903-0788
RT. 137, China Road
Winslow, Maine 04901

Administrative Offices
Phone: 207-873-7711
Fax: 207-873-7022

Customer Service
Phone: 1-800-244-8378
Fax: 207-873-7022

TECHNICAL PRODUCT INFORMATION

D/E NEUTRALIZING AGAR

Catalog No:	Description
P4005	Contact Plate
P1285	Standard Petri Dish

D/E NEUTRALIZING BROTH

Catalog No:	Description
T1451	9 mL
T1460	3 mL
T1461	25 mL
T1590	9 mL
T1595	9.9 mL
T1597	10 mL w/Swab

INTENDED USE:

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

D/E Neutralizing Agar has the ability to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals and is used for environmental sampling for detection and enumeration of microorganisms present on surfaces of sanitary importance. D/E Neutralizing Agar and D/E Neutralizing Broth are especially suited for environmental sampling where neutralization of the chemical is important to determine its bactericidal activity. A strongly bacteriostatic substance may contain bacteria held in bacteriostasis but which may still be able to cause infection. The media will neutralize a broad spectrum of antiseptic and disinfectant chemicals including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials (Merthiolate), formaldehyde and gluteraldehyde. D/E Neutralizing Broth Base has the same formula as D/E Neutralizing Broth but does not contain the neutralizing components.

Total neutralization of disinfectants is critical, as residues can result in a false negative (no-growth) test. D/E Neutralizing Agar effectively neutralize the inhibitory action of disinfectant carryover, allowing differentiation between bacteriostasis and true bactericidal action of disinfectant chemicals. D/E Neutralizing agar is recommended for use in disinfectant evaluations, environmental sampling (swab and contact plate methods), and testing of water-miscible cosmetics.

PRINCIPLES:

Peptone, yeast extract and dextrose are nutrient sources; peptone also provides nitrogenous compounds and essential amino acids. Yeast extract is a vitamin B-complex source and dextrose is an energy source as well. The five neutralizers in this medium, sodium thioglycollate, sodium thiosulfate, sodium bisulfite, lecithin and Polysorbate 80, inactivate a variety of disinfectant and antiseptic chemicals. Bromcresol purple is an indicator for dextrose utilization.

FORMULA:

D/E Neutralizing Broth

Component (per liter of purified water)	Amount
Tryptone	5.0 g
Yeast Extract	2.5 g
Dextrose	10.0 g
Sodium Thioglycollate	1.0 g
Sodium Thiosulfate	6.0 g
Sodium Bisulfite	2.5 g
Lecithin (Soybean)	7.0 g
Polysorbate 80	5.0 g
Brom Cresol Purple	0.02 g

Final pH: 7.6 ± 0.2 @ 25°C.

D/E Neutralizing Agar also contains 15.0 g/L of agar

PROCEDURE:
For testing disinfectants, prepare two sets of test tubes, one containing 9 mL of sterile D/E Neutralizing Broth and another set containing 9 mL amounts of D/E Neutralizing Broth Base. Add 1 mL of disinfectant solution to each tube, mix thoroughly and let stand for 15 minutes. The disinfectant may require diluting to obtain the neutralized concentration. Inoculate the tubes with 0.1 mL of a 1:100,000 dilution of over-night broth cultures.

Incubate both sets of tubes for 48 hours at 37°C and observe growth, which is indicated by a color change from purple to yellow or by the formation of a pellicle. Growth in D/E Neutralizing Broth with no growth in D/E Neutralizing Broth Base indicates neutralizing of the disinfectant and a possible bacteriostatic substance. To determine if viable organisms are present and to indicate bactericidal activity, inoculate samples from the broth tubes into plates of D/E Neutralizing Agar. Incubate for 48 hours at 37°C. Positive growth from negative tubes of D/E Neutralizing Broth Base indicates a bacteriostatic substance whereas negative growth indicates a bactericidal disinfectant. All positive broth tubes should show growth on the D/E Neutralizing Agar Plates.

A disk plate method may also be used for testing disinfectants using D/E Neutralizing Agar. In this procedure, pour plates are prepared with inocula from the test cultures as needed, depending on the disinfectant to be tested. Prepare plates (100 x 15 mm) by aseptically dispensing 20 mL of inoculated medium into each plate. Place one ½ inch sterile white filter paper disk for each control and test substance on the plates and dispense 0.1 mL control and test substance solution on the disks. Also prepare plates of standard methods agar in the same manner. Incubate the plates for 40 to 48 hours at 35 ± 2°C. Zones of inhibition occurring around disks on the standard methods agar plates with no zones occurring on the D/E Neutralizing Agar Plates indicates neutralizing of the disinfectant by D/E Neutralizing Agar.

The following control disinfectants may be used in the test procedure: chlorine 2% (chlorine bleach), formaldehyde 2%, gluteraldehyde 1%, iodine 2%, merthiolate 1/1000, phenol 2%, and quaternary ammonium compounds 1/750. One or more of these controls may be used depending on the types of disinfectant to be tested.[1]

STORAGE:
Store prepared media, Agar & Broth at 2 to 8°C. Do not use if media appears deteriorated or use beyond media expiration date.

QUALITY CONTROL:

Organism	Growth
ATCC# 25922 *Escherichia coli*	Good to excellent
ATCC# 27853 *Pseudomonas aeruginosa*	Good to excellent
ATCC# 14028 *Salmonella typhimurium*	Good to excellent
ATCC# 25923 *Staphylococcus aureus*	Good to excellent

REFERENCES:

1. Difco & BBL Manual, Manual of Microbiological Culture Media, 2009, Page 172
2. Acumedia 7375 D/E Neutralizing Agar Product Information Sheet Rev 3, February 2011