PRODUCT SPECIFICATION SHEET

Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing) (DM791)

Intended Use

Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing) (DM791) is recommended for disinfectant testing, where neutralization of antiseptics and disinfectants is important for determining its bactericidal activity.

Product Summary and Explanation

Dey-Engley Neutralizing Agar is formulated as per the procedure described by Engley and Dey to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals, including quaternary ammonium compounds, phenolics, iodine, chlorine preparations, mercurials, formaldehyde, and glutaraldehyde.[1] A strongly bacteriostatic substance inhibits the growth and reproduction of bacteria without killing them. These bacteria hold the ability to cause infection under favourable conditions. D/E Neutralizing media neutralizes higher concentrations of residual antimicrobials as compared with other standard neutralizing formulations, such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[2,3] Complete neutralization of disinfectants is crucial and disinfectant residues can result in a false negative (no-growth) test. D/E Neutralizing Agar allows differentiation between bacteriostasis and true bactericidal action of disinfectant chemicals by effectively neutralizing the inhibitory action of disinfectant carryover.[4,5] This is a significant characteristic to consider when evaluating a disinfectant. D/E Neutralizing Agar is also recommended for use in disinfectant evaluations, environmental sampling (swab and contact plate methods), and testing of water- miscible cosmetics.[6]

Principles of the Procedure

Dey-Engley Neutralizing Agar contains casein enzymic hydrolysate which provide essential nutrients for metabolism. Dextrose is an energy and carbon source. Yeast extract is also a rich source of vitamin B-complex. The present formulation incorporates neutralizing substances for almost all the active products used as antiseptics and disinfectants. Sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurials; sodium thiosulfate neutralizes iodine and chlorine,[1] lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface-active agent, neutralizes substituted phenolics.[7-10] Bromocresol purple is an indicator for dextrose utilization. Due to the high concentration of lecithin, bromocresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow.

Formula / Liter

Ingredients	Gms / Liter
Part A	
Casein enzymic hydrolysate	5.00
Yeast extract	2.50
Dextrose	10.00
Sodium thiosulphate	6.00
Sodium thioglycollate	1.00
Sodium bisulphite	2.50
Bromocresol purple	0.02
Agar	15.00
Total	**42.02**
Part B	
Polysorbate 80	5.00
Part C	
Lecithin	7.00
Final pH : 7.6 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 7 gm Part C separately in 50ml distilled water and boil at 100 °C for 10 minutes
2. Suspend 42.02 grams of part A + 5 grams of part B in 950 ml distilled water and add part C 50ml.
3. Heat to boiling to dissolve the medium completely.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle. Mix well and dispense as desired.

Quality Control Specifications

Dehydrated Appearance	Part A: Light yellow to bluish grey homogeneous free flowing powder Part B: Yellow coloured solution
Prepared Medium	Purple coloured, opalescent gel forms in Petri plates
Reaction of 5.4% Solution	pH : 7.6 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35 - 37°C for 40 - 48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Escherichia coli ATCC 25922*	50 -100	good-luxuriant	>=70%
2.	*Pseudomonas aeruginosa ATCC 27853*	50 -100	good-luxuriant	>=70%
3.	*Salmonella Typhimurium ATCC 14028*	50 -100	good-luxuriant	>=70%
4.	*Staphylococcus aureus ATCC 25923*	50 -100	good-luxuriant	>=70%
5.	*Bacillus subtilis ATCC 6633*	50 -100	good-luxuriant	>=70%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Dey-Engley Neutralizing Agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden.
2. Selected surfaces are sampled by firmly pressing the agar medium against the test area.
3. Hold the plates with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample.
4. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult.
5. Slightly curved surfaces may be sampled with a rolling motion.
6. Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.
7. Neutralization Test: Growth in Neutralizing Broth and no growth in Neutralizing Broth Base indicate neutralization of disinfectant. To check bactericidal activity, both broth tubes are inoculated on D/E Neutralizing Agar. Positive growth from negative tubes of Neutralizing Broth Base indicates bacteriostatic substance while negative growth indicates a bactericidal disinfectant. All positive tubes should show growth on Dey-Engley Neutralizing Agar. The control disinfectants used in test procedure are 2% chlorine, 2% formaldehyde, 1% glutaraldehyde, 2% iodine, 2% phenol, 1/750 quaternary ammonium compounds, 1/1000 mercurials etc.
8. Refer to appropriate references for standard test procedures.

Results

1. After incubation, count visible colonies on plated medium. Counting of plates containing a profusion of growth can lead to considerable error. A basic decision to be made is whether distinct colony margins can be observed. Spreading colonies should be counted as one but care taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-coloured colonies and halo-type spreaders. It is generally agreed that 200 colonies is the approximate maximum that can be counted on these plates.

2. Colony counts may be recorded by:
   a) Simply keeping individual counts.
   b) Number of viable particles per square foot (agar area of **RODAC** plates is 3.97 square inches).
   c) Means and standard deviations.
3. Subculture the colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examinations of organism smears.
4. Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing)
Product Code : DM791
Available Pack sizes : 500gm

References

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4. Dey, B. P., and F. B. Engley, Jr. 1994. Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods. 19:51-58.
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7. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed. American Public Health Association, Washington, D.C.
8. Quisno R.A., Gibby I.W., and Foter M.J., 1946, Am. J. Phar., 118:320.
9. Erlandson A. L., and Lawrence C. A., 1953, Science 118:274.
10. Brummer B., 1976, Appl. Environ. Microbiol., 32:80.

Further Information

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