



Antibiotic Assay Medium No. 32

M1141

Antibiotic Assay Medium No. 32 is recommended for preparing inoculum of *Bacillus subtilis* to be used as a test organism for assaying Dihydrostreptomycin and Vancomycin by plate assay method.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue (Peptone)	6.000
Casein enzymic hydrolysate	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Manganese sulphate	0.300
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.8 gms in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilise by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice : Recommended for the microbiological assay of Dihydrostreptomycin and Vancomycin

Principle And Interpretation

This medium is formulated in accordance to FDA (1) and is a modification of Antibiotic assay Medium No.1. This medium is used to develop inoculum of *Bacillus subtilis* for antibiotic assay.

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by peptic digest of animal tissue, pancreatic digest of casein, yeast extract and beef extract. Dextrose in the medium serves as the carbon source for stimulating the growth of the test microorganism. Manganese sulphate in this medium facilitates the sporulation and growth of *Bacillus subtilis* (2,3,4), which is generally used as test organisms for plate assay of Dihydrostreptomycin and Vancomycin.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.08% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

M1141: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	≥70 %	Dihydrostreptomycin, Vancomycin

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label

Reference

1. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April). ,,
2. Vasantha & Freese, 1979, J.Gen.Microbiol. 112:329-336
3. Charney, J., Fisher, W.P. and Hegarty, C.P. 1951. J. Bacteriol. 62:145.
4. Curran, H.R. and Evans, F.R. 1954. J. Bacteriol. 67: 489.

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