



Antibiotic Assay Medium No. 32

MU1141

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

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Pancreatic digest of casein	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±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.8 gms in 1000ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilise by autoclaving at 15lbs 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 USP and FDA (1,2) 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 peptone, pancreatic digest of casein, yeast 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* (3,4,5), which is generally used as test organisms for plate assay of Dihydrostreptomycin and Vancomycin .

Quality Control

Appearance

Cream to yellow coloured 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 . pH : 6.6±0.1

pH

6.50-6.70

Cultural Response

MU1141: Cultural characteristics observed after an incubation at 32-35°C for 5 days.

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. United States Pharmacopoeia 2011 , US Pharmacopoeial Convention, Inc., Rockville, MD.
2. 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).
3. Vasantha & Freese, 1979, J.Gen.Microbiol. 112:329-336
4. Charney, J., Fisher, W.P. and Hegarty, C.P. 1951. J. Bacteriol. 62:145.
5. Curran, H.R. and Evans, F.R. 1954. J. Bacteriol. 67: 489

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