



Antibiotic Assay Medium No. 4

MU140

Antibiotic Assay Medium No. 4 is used for detection of Penicillin in milk samples and in microbiological assay of different antibiotics in accordance with United States Pharmacopoeia.

Composition**

| Ingredients | Gms / Litre |
|-----------------------------------|-------------|
| Peptone | 6.000 |
| Yeast extract | 3.000 |
| Beef extract | 1.500 |
| Dextrose | 1.000 |
| Agar | 15.000 |
| pH after sterilization (at 25°C) | 6.6±0.1 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.5 grams in 1000 ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

This dehydrated culture medium is suitable for plate counts in pharmaceutical and related products and for the microbial assay and detection of antibiotics like penicillin in milk. This medium is formulated in accordance to the specifications and procedures listed by the Food and Drug Administration and USP (1, 2). This medium is identical numerically with name assigned by Grove and Randall (3),,

Peptone, yeast and beef extract provide nutritional requirement for growth of the indicator organisms like *Bacillus stearothermophilus*, *Micrococcus luteus*. This medium is similar to Antibiotic assay medium no. 2 except for the additional ingredient dextrose. Dextrose in the medium serves as an easily available source of carbon stimulating luxuriant growth of the test organisms.

Generally presence of penicillin in milk is detected by the cylinder plate method, using *Micrococcus luteus* as the test organism, and by paper disk method, using *Bacillus stearothermophilus*. The cylinder plate method is recommended as the standard for quantification of β -lactam residues. A description of the cylinder plate method for detecting penicillin in dry powdered milk is given by Kramer et al. (4). The same basic procedure is also recommended to the assay of penicillin in fluid milk.

Freshly prepared plates should be used for antibiotic assays. The use of this medium assures well defined zones of the test organism. All conditions in the microbiological assay must be controlled carefully. The use of standard culture medium in the test is one of the important steps for obtaining good results.

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 2.65% w/v aqueous solution (after sterilization). pH : 6.6±0.1

pH

6.50-6.70

Cultural Response

MU140: Growth Promotion is carried out in accordance with USP. Cultural characteristics observed after an incubation at 32-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar.

| Organism | Inoculum (CFU) | Growth | Recovery | Incubation temperature | Incubation period |
|--|----------------|----------------|----------|------------------------|-------------------|
| Cultural Response | | | | | |
| <i>Micrococcus luteus</i> ATCC 10240 | 50-100 | good-luxuriant | >=50% | 32-35°C | 18-24 hours |
| <i>Bacillus stearothermophilus</i> ATCC 7953 | 50-100 | good-luxuriant | >=50% | 55°C | 18-24 hours |

Storage and Shelf Life

Store below 30°C 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 1).
2. United States Pharmacopoeia/National Formulary 2009, US Pharmacopoeial Convention, Inc., Rockville, MD.
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
4. Kramer, J., G.G. Carter, B. Arret, J. Wilner, W.W. Wright, and A. Kirshbaum. 1968. Antibiotic residues in milk, dairy products and animal tissues: methods, reports and protocols. Food and Drug Administration, Washington, DC.

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