



## Antibiotic Assay Medium No. 4 (Yeast Beef Agar)

M140

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) is used for detection of Penicillin-G in milk samples.

### Composition\*\*

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

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 26.5 grams in 1000 ml of 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

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) 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 (1). This medium is identical numerically with name assigned by Grove and Randall (2).

Peptic digest of animal tissue, yeast and beef extract provides 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 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 a paper disk method, using *Bacillus stearothermophilus* . The cylinder plate method is recommended as the standard for quantification of  $\beta$ -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 in 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 step for obtaining good results.

### 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 2.65% w/v aqueous solution at 25°C. pH : 6.6±0.2

#### pH

6.40-6.80

### Cultural Response

M140: Cultural characteristics observed after an incubation at 55°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Bacillus stearothermophilus</i> ATCC 7953	50-100	good-luxuriant	>=50%
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	>=50%

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C in tightly closed container . 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. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
3. 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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