



## Antibiotic Assay Medium No.9

MU147

Antibiotic Assay Medium No.9 is used as Base layer for plate assay of Carbenicillin , Colistimethate sodium and Polymyxin B in accordance with United States Pharmacopoeia.

### Composition\*\*

Ingredients	Gms / Litre
Pancreatic digest of casein	17.000
Papaic digest of soybean	3.000
Dextrose	2.500
Sodium chloride	5.000
Dibasic potassium phosphate	2.500
Agar	20.000
pH after sterilization	7.2±0.1

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

### Directions

Suspend 50 grams in 1000 ml 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 medium is widely recommended for assay of Polymyxin B, Colistimethate sodium and Colistin using *Bordetella bronchiseptica* as test organisms. Carbenicillin assay is also performed using this medium with *Pseudomonas aeruginosa*. The medium is formulated in accordance with USP and CFR (1,2) and numerically identical with the name assigned by Groove and Randall (3).

Pancreatic digest of casein and papaic digest of soybean meal serves as source for essential nutrients. Dextrose stimulates the growth by providing carbon and energy. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium in the medium. Agar concentration provides control over the diffusion activity of Polymyxin B antibiotics and provides solid substratum to support the seed agar layer.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized culture can be overlaid. Even distribution of the layer is important.

### Quality Control

#### Appearance

Cream to yellow coloured homogeneous free flowing powder

#### Gelling

Firm, comparable with 2.0% agar gel.

#### Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates.

#### pH

7.10-7.30

#### Cultural Response

MU147: Cultural characteristics observed after an incubation at 36-37.5°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
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<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	luxuriant	>=50%	Polymyxin B, Colistimethate sodium, Colistin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	>=70%	Carbenicillin

### 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, (April1).
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.

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