Antibiotic Assay Medium No. 6

M223

Antibiotic Assay Medium No. 6 is used for induction of spore production in *Bacillus subtilis* strains used in antibiotic assays.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dextrose	2.500
Dipotassium phosphate	2.500
Manganese sulphate	0.030
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.03 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (2). These media are prepared as per FDA (3).

Casein enzymic hydrolysate and papaic digest of soyabean meal provides the nutrients and growth factors. Dextrose provides as energy source. Dipotassium phosphate provides the buffering system. Manganese sulphate helps in the early initiation of *Bacillus* species.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured clear solution may contain slight precipitate.

Reaction
Reaction of 3.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M223: Cultural characteristics observed after an incubation at different temperatures for 6 days.

Organism	Inoculum (CFU)	Growth	Incubated at	Spores
Bacillus cereus ATCC 10876	50-100	luxuriant	30°C	positive
Bacillus stearothermophilus ATCC 7953	50-100	luxuriant	55°C	positive
Bacillus subtilis ATCC 6633	50-100	luxuriant	35°C	positive
Bacillus pumilus ATCC 14884	50-100	luxuriant	35°C	positive

Storage and Shelf Life

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

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc, New York.
2. Schmidt and Moyer, 1944; J. Bact, 47:199.
3. 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 pg 242-259 (April 1).

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