



## Antibiotic Assay Medium No.35

M798

Antibiotic Assay Medium No. 35 is used for the microbiological assay of Bleomycin using *Mycobacterium smegmatis* .

### Composition\*\*

| Ingredients                              | Gms / Litre |
|--|-------------|
| Peptic digest of animal tissue (Peptone) | 10.000      |
| Beef extract                             | 10.000      |
| Sodium chloride                          | 3.000       |
| Agar                                     | 17.000      |
| Final pH ( at 25°C)                      | 7.0±0.2     |

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

### Directions

Suspend 40 grams in 1000 ml distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

*Advice : Recommended for the microbiological assay of Bleomycin*

### Principle And Interpretation

This medium is formulated in accordance to CFR (1). This medium is employed widely as base agar for agar diffusion assay of Bleomycin using *Mycobacterium smegmatis* . The nutrients essential for growth of test organism is provided by peptic digest of animal tissue and beef extract in this medium. Agar provides excellent solid substratum for support and overlaying of seed agar, for the assay of Bleomycin. Addition of glycerol is important for provision of carbon to the test organism.

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 homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.7% agar gel.

#### Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 4.0% w/v aqueous solution containing 1% glycerol at 25°C. pH : 7.0±0.2

#### pH

6.80-7.20

#### Cultural Response

M798: Cultural characteristics observed after an incubation at 36-37.5 for 18-48 hours.

| Organism                                   | Inoculum (CFU) | Growth    | Recovery | Antibiotics assayed |
|--|----------------|-----------|----------|---------------------|
| <i>Mycobacterium smegmatis</i><br>ATCC 607 | 50-100         | luxuriant | ≥50%     | Bleomycin           |

### 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. 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).

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