



## Antibiotic Assay Medium G (ME553/M553B)

MAP553

### Intended Use:

Recommended for the microbiological assay of Bleomycin using *Mycobacterium smegmatis*, as a test organism in accordance with EP/BP.

### Composition\*\*

Ingredients	g / L
Peptone	10.000
HM extract #	10.000
Sodium chloride	3.000
Agar	15.000
pH after sterilization	7.0±0.1

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

# Equivalent to Meat extract

### Directions

Suspend 38.0 grams in 1000 ml water R/purified/distilled water containing 10 grams glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates or as desired.

Advice: Recommended for the microbiological assay of Bleomycin sulphate CRS.

### Principle And Interpretation

This medium is formulated in accordance to European Pharmacopoeia and British Pharmacopoeia (1,2). This medium is employed widely as base agar for agar diffusion assay of Bleomycin using *Mycobacterium smegmatis*. It is also used for preparing the inoculum of *Mycobacterium smegmatis* for assay.

The nutrients essential for growth of test organism is provided by peptone and HM extract in this medium. Agar provides excellent solid substratum for support and over layering of seed agar, for the assay of Bleomycin. Addition of glycerol is important for provision of carbon to the test organism.

### Type of specimen

Antibiotics as per EP & BP

### Specimen Collection and Handling

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. After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations

1. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Freshly prepared plates should be used for antibiotic assays.

### Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Cream to yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

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

### Reaction

After sterilization, reaction of 3.8% w/v aqueous solution containing 1% glycerol. pH : 7.0±0.1

### pH

6.90-7.10

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotic assayed
<i>Mycobacterium smegmatis</i> ATCC 607	50-100	good-luxuriant	≥70%	Bleomycin sulphate

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

## Reference

1. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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