# Antibiotic Assay Medium E

**MAP1347**

## (ME1347/M1347B)

## Intended Use:

Recommended for microbiological assay of Neomycin sulphate and Framycetin sulphate using *Bacillus spizizenii* and *Bacillus pumilus* in accordance with EP/BP.

## Composition**

| Ingredients | g/ L |
|---|---|
| Peptone | 5.000 |
| Disodium hydrogen phosphate,12$H_2O$ | 26.900 |
| HM extract # | 3.000 |
| Agar | 10.000 |
| pH after sterilization | 7.9±0.1 |

**Formula adjusted, standardized to suit performance parameters
# Equivalent to Meat extract

## Directions

Suspend 28.67 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml R-water/purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the European pharmacopoeia for the antibiotic assayed.

*Advice : Recommended for the microbiological assay of Framycetin Sulphate and Neomycin sulphate.*

## Principle And Interpretation

This medium is formulated in accordance to European Pharmacopoeia (1) and British Pharmacopoeia (2).This medium is widely used for as seed agar in plate assay of Framycetin sulphate and Neomycin sulphate using *Bacillus spizizenii* and/ or *Bacillus pumilus* as test organism.

Peptone and HM extract extract supply nutrients essential for microbial growth. Phosphates are incorporated in the medium to provide good buffering action. The low concentration of agar facilitates proper diffusion of antibiotic in the seed agar. Freshly prepared plates should be used for antibiotic assays.

## Type of specimen

Antibiotics

## Specimen Collection and Handling

Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. Zones of inhibition around the antibiotic are then measured. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results. 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 1.0% Agar gel.

**Colour and Clarity of prepared medium**

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

**pH**

7.80-8.00

**Cultural Response**

Cultural characteristics observed after an incubation at 30-37°C for 18-24 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Antibiotics assayed |
|---|---|---|---|---|
| *Bacillus pumilus* NCTC 8241 | 50-100 | luxuriant | >=70% | Framycetin sulphate, Neomycin sulphate |
| ** *Bacillus spizizenii* ATCC 6633 (00003*) | 50-100 | luxuriant | >=70% | Neomycin sulphate and Framycetin sulphate |

Key : *Corresponding WDCM numbers. **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

## 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.

Revision : 01/2024