



## Antibiotic Assay Medium C (ME555/M555B)

MAP555

### Intended Use:

Used as the broth medium in turbidimetric assay of a wide variety of antibiotics in accordance with EP/BP.

### Composition\*\*

Ingredients	g / L
Peptone	6.000
HM peptone B #	1.500
Yeast extract	3.000
Sodium chloride	3.500
Glucose monohydrate	1.000
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
pH after sterilization	* 7.0±0.1

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

\*While assaying Josamycin & Josamycin propionate adjust the pH to 8.0 ±0.1

# Equivalent to Beef extract

### Directions

Suspend 19.9 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml R-water/purified/distilled water. Heat with frequent agitation 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 EP for the antibiotic assayed.

Note: *Adjust the medium pH to 8.0 ± 0.1 for assaying Josamycin & Josamycin propionate.*

Advice: *Recommended for the Microbiological assay of Colistimethate sodium, Colistin sulfate, Framycetin sulphate, Gentamicin sulphate, Gramicidin, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Rifamycin sodium, Spiramycin, Streptomycin sulphate, Tylosin, Tylosin tartrate, Tyrothricin and Vancomycin hydrochloride in accordance with E.P.*

### Principle And Interpretation

This medium is used in turbidimetric assay of several antibiotics. The composition of the medium is in accordance to the specifications detailed in the European Pharmacopoeia and British Pharmacopoeia (1,2). Turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than comparable agar diffusion procedures (3).

Peptone, HM peptone B and yeast extract provide essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic equilibrium while phosphates are incorporated in the medium to provide good buffering action. Glucose monohydrate serves as the carbon and energy source for faster growth.

Turbidimetric antibiotic assay is based on the change or inhibition of growth of a test microorganisms in a liquid medium containing a uniform concentration of an antibiotic (4). Use of this method is appropriate only when test samples are clear.

### Type of specimen

Antibiotics as per EP and BP

### Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines (1,2).

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. Freshly prepared plates should be used for antibiotic assays.
2. Use of this method is appropriate only when test samples are clear.

### 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

#### Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

#### pH

6.90-7.10

#### Growth promotion test

In accordance with EP/BP

#### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Serial dilution with
<i>Escherichia coli</i> ATCC 9637	50-100	luxuriant	Colistimethate sodium, Colistin sulfate
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	Rifamycin sodium
<i>Enterococcus hirae</i> ATCC 10541 (00011*)	50-100	luxuriant	Gramicidin, Tyrothricin
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	Streptomycin sulphate
<i>Staphylococcus aureus</i> ATCC 6538P	50-100	luxuriant	Framycetin sulphate, Genatamicin sulphate, Gramicidin, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Spiramycin; For Josamycin & Josamycin propionate-adjust the pH of the medium to 8.0 ± 0.1, For Vancomycin hydrochloride incubate at 37-39°C.
<i>Staphylococcus aureus</i> ATCC 9144 (00035*)	50-100	luxuriant	Tylosin, Tylosin tartarate

Key : \*Corresponding WDCM numbers.

### 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 (4,5).

## 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. Ripper RA. Some principles of microbiological turbidimetric assays of antibiotics. J Assoc Off Anal Chem.1979 62(4):951-6.Chapin-Robertson and Edberg, 1991, Measurement of Antibiotics in Human Body fluids: Techniques and significance. Antibiotics in Laboratory medicine, New York pp 305
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
5. 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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### Disclaimer :

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