TM 1749 – ANTIBIOTIC ASSAY MEDIUM H (as per IP)

INTENDED USE

For microbiological plate assay of Carbenicillin and Polymyxin-B.

PRODUCT SUMMARY AND EXPLANATION

This medium is used as seed agar for assay of Carbenicillin, Colistimethate sodium, Colistin sulphate and Polymyxin B. The medium composition is in accordance to IP and CFR and numerically identical with the name assigned by Groove and Randall.

Polymyxin are reported to have slow diffusion in agar giving smaller zone of inhibition. Hence the reduced agar concentration (1.2%) in this medium improves the diffusion of polymyxin in the medium. Polysorbate 80 are reported to function synergistically with polymyxin on spheroplasts of *Pseudomonas aeruginosa*. Polysorbate 80 enhances the penetration of Polymyxin to the cytoplasmic membrane and hence is an appropriate ingredient in the medium used for assay of Polymyxin. Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important step for the good results.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	17.000
Soya peptone	3.000
Dextrose (Glucose)	2.500
Sodium Chloride	5.000
Dipotassium hydrogen phosphate	2.500
Agar	12.000

PRINCIPLE

Combination of tryptone and soya peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients for the growth of test organisms. Natural soya sugars enhance microbial growth. Dextrose (Glucose) provides the carbon source, enhances the growth of test organism. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium.

INSTRUCTION FOR USE

- Dissolve 42 grams in 1000 ml purified / distilled water containing 10 ml of Polysorbate 80.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Medium amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.2±0.1

INTERPRETATION

Cultural characteristics observed after incubation.

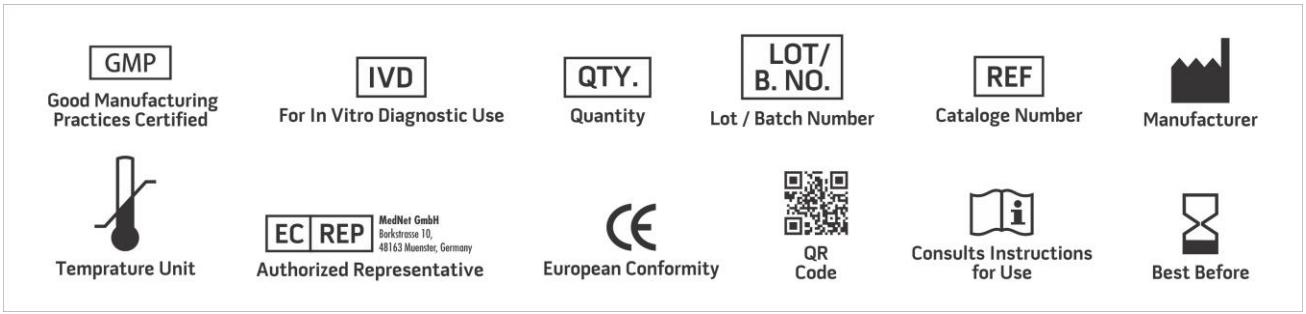
Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Antibiotics assayed	Incubation Temperature	Incubation Period
Bordetella bronchiseptica	4617	50-100	Luxuriant	>=70%	Polymyxin B, Colistimethate sodium, Colistin sulphate	32-35°C	24 Hours
Pseudomonas aeruginosa	25619	50-100	Luxuriant	>=70%	Carbenicillin	36-37.5°C	24 Hours
Escherichia coli	10536	50-100	Luxuriant	>=70%	Colistimethate sodium, Colistin sulphate	35-39°C	24 Hours

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
1.Barry, 1991, Procedure and theoretical considerations for testing antimicrobial agents in agar media.Antibiotics in Laboratory medicine, New York pp 3
2. Brown & Winsley, 1968. J Gen Microbiol. 1968 50(3) Supplix.
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
4. Indian Pharmacopeia 2014, Ministry of Health and Family welfare, Government of India, New Delhi
5. 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, (April1).



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**

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