

TECHNICAL DATA SHEET

Antibiotic Assay Medium No.32

Principle

The media is composed according to the USP, recommended as antibiotic assay medium. It is composed of peptone, casein peptone, yeast extract, meat extract (equivalent to beef extract), dextrose, manganese sulphate and agar. Peptone, casein peptone, yeast extract and meat peptone provide nitrogen, carbon, long chain amino acids, vitamins and essential nutrients for the growth of microorganisms. Dextrose serves as energy source. Manganese sulphate in this medium facilitates the sporulation and growth of *Bacillus subtilis*. Agar is solidifying agent.

Use: For microbiological assay of Dihydrostreptomycin and Vancomycin by preparing inoculum of *Bacillus subtilis* ATCC 6633

Contents*

| Ingredients | Gram/Litre |
|----------------------------------|------------|
| Peptone | 6.000 |
| Casein peptone | 4.000 |
| Yeast Extract | 3.000 |
| Meat Extract# | 1.500 |
| Dextrose | 1.000 |
| Manganese sulphate | 0.300 |
| Agar | 15.000 |
| pH at 25°C (after sterilization) | 6.6 ±0.1 |

* Formula adjusted for optimum performance and parameters # Equivalent of Beef Extract

Directions: Dissolve 30.80 grams in 1000 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 min, cool it to 42-45 °C, and distribute aseptically in sterile petri plates. Ensure complete solidification and inoculate test organisms aseptically.

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Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

| | |
|-----------------------------------|--|
| Appearance | Light beige colored free flowing, homogeneous powder |
| Reaction of 3.08% solution | 6.6 ±0.1 at 25 °C |
| pH | 6.40- 6.80 |
| Gelling | Firm comparable with 1.5% agar gel |
| Color and clarity of ready medium | Light amber colored opalescent gel |
| Growth Promotion properties | Best at ≤ 100 CFU at 33-37 °C for 18-72 h |
| Indicative properties | Optimum at ≤ 100 CFU at 33-37 °C for 18-48 h |
| Negative control | Performed using sterile distilled water |

Different Microbial Response: Cultural characteristics observed after incubation at 35±2°C for 5 days.

| Organism | ATCC | Inoculum (CFU) | Growth | Recovery | Antibiotic assayed |
|--------------------------|------|----------------|-----------|----------|------------------------------------|
| <i>Bacillus subtilis</i> | 6633 | 50-100 | Luxuriant | ≥ 70% | Dihydrostreptomycin and Vancomycin |

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

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Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
2. *Difco Manual* (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. *The United States Pharmacopoeia*, (2014), The United States Pharmacopeial Convention. 12601 Twinbrook Parkway, Rockville, MD 20852.

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