Instructions for Use

REF - BM4712 **Antibiotic Medium No.32 (USP)**

Bottled Media

1. Intended Use

BM4712 Antibiotic Medium No.32 (USP) is used for the determination of the activity of antibiotics in pharmaceutical and other products.

2. Composition*

Ingredient	g/L
Meat peptone	6.0
Yeast extract	3.0
Beef extract	1.5
Bacteriological agar	15.0
Tryptone	4.0
Glucose	1.0
Manganese sulphate	0.1

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium supports the growth of a wide range of organisms. Antibiotic Medium No.32 is a modification of Antibiotic Medium No.1 (E&O BM4710) suitable for the assay of dihydrostreptomycin, vancomycin, and other antibiotics by the plate assay method. Inhibition zones on the medium resulting from application of a defined quantity of the product are compared to zones produced by antibiotic standards. The medium conforms to the requirements of the United States Pharmacopeia (USP) [(1)].

4. Principle

Nutrients and growth factors are supplied by meat peptone, yeast extract, beef extract, and tryptone. Glucose is a carbon source.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Straw liquid	6.6 ± 0.2

6. Materials Provided

BM4712 Antibiotic Medium No.32 (USP) can be provided in 10 x 100ml in 125ml Sirop Bottles (product code: BM4712-R125-100). Each bottle is ink-jet printed or labelled with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

8. Specimens

For testing individual isolated colonies for antimicrobial susceptibility testing by the disk diffusion method as described by Bauer-Kirby [(2)]. Use a sterile loop or cotton swab to pick colonies from an overnight culture on non-selective media. If possible, use several morphologically similar colonies to avoid selecting an atypical variant.

Suspend in saline and mix to an even turbidity. Adjust the density of the suspension to 0.5 McFarland by adding saline or more bacteria. Preferably use a photometric device to measure the turbidity.

Optimally, use the inoculum suspension within 15 minutes of preparation and always within 60 minutes.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Boil agar until it has entirely melted. Mix, and allow to cool to approximately 45°C, pour plates and allow to set.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Dip a sterile cotton swab into the inoculum suspension. For Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

Spread the inoculum evenly over the entire surface by swabbing in three directions or by using a plate rotator. For Gram-positive bacteria, take particular care to ensure that there are no gaps between streaks. When inoculating several agar plates with the same inoculum, dip the cotton swab into the suspension for each agar plate.

Apply disks within 15 min of inoculation. Disks must have even contact with the agar surface. The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application at 35 ± 2°C for 16-18 hours.

Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30cm from the eye. Measure zone diameters to the nearest millimetre with a calibrated ruler or calibrated callipers. If an automated zone reader is used, it must be calibrated to manual reading.

10. Quality Control

Organism	Incubation	Result (Specificity)
B. subtilis (NCTC 10400)	30-35°C for 18-24 hours	Growth: Opaque/grey colonies
S. aureus (NCTC 10788)	30-35°C for 18-24 hours	Growth: Yellow colonies
E. coli (NCTC 12923)	30-35°C for 18-24 hours	Growth: Cream colonies

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify BM4712 Antibiotic Medium No.32 (USP) performance, samples were tested to assess colony morphology and recovery when incubated at 30-35°C for 18-24 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538). Therefore, it can be concluded that BM4712 Antibiotic Medium No.32 (USP), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Due to natural variation, some strains may grow poorly on this medium.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM4712 in the original bottle with the lid tightly closed at between 15 and 30°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
2. Hudzicki J (2009) Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiol.

Version History*

001 27/08/24 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

EC REP
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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place