

Instructions for Use

REF - BM5500

Non-Nutrient Agar

Bottled Media

1. Intended Use

For *in vitro* diagnostic use only. BM5500 Non-Nutrient Agar is a nutrient-deplete medium recommended for the detection of free-living pathogenic *Acanthamoeba* and *Naegleria* spp. cysts, trophozoites, and flagellates in tissue, soil, or water samples.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Bacteriological agar	15.0
Disodium hydrogen phosphate	0.142
Potassium dihydrogen phosphate	0.136
Sodium chloride	0.12
Magnesium sulphate	0.004
Calcium chloride	0.004

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Non-Nutrient Agar is based on Page's Saline solidified with 1.5% agar. Page's Saline (Amoeba Saline) was developed by Page in the 1960s ⁽¹⁾ to provide a suitable environment for the isolation and cultivation of amoebae, particularly free-living amoebae. These amoebae are commonly found in diverse habitats such as soil, water bodies, and human tissues.

4. Principle

Non-Nutrient Agar has a balanced salt content that mimics the osmotic environment found in the body fluids of animals making it suitable for the cultivation and study of a wide range of microorganisms. Agar is the solidifying agent.

5. Physical Characteristics

	<u>Appearance and Colour</u>	<u>pH</u>
Medium	Opalescent gel	6.9 ± 0.3

6. Materials Provided

BM5500 Non-Nutrient Agar is available as 50 x 3ml in Thin Wall Bijous (product code: BM5500-M003-3). 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

BM5500 Non-Nutrient Agar is suitable for the testing of the following specimens:

- Clinical Specimens: Can include corneal scrapings or eye swabs, respiratory specimens, cerebrospinal fluid, genital specimens, and tissue biopsies.
- Food and Environmental: products for human consumption, animal feed and environmental samples

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

Transfer a portion of the collected sample into a sterile container or culture dish containing Page's Saline (Amoeba Saline) (E&O BM4020). The volume of the sample to be added will depend on the specific experimental requirements or protocols being followed. Refer to relevant guidelines.

Incubate sample in optimal growth conditions for the target organism. The incubation temperature and duration will vary depending on the specific amoeba species being targeted.

Periodically examine the culture for the presence of amoebae under a microscope for up to two weeks. Monitor for the characteristic morphology and motility of amoebae. Amoebae can be identified based on their distinct shape, pseudopodia (false feet) extensions, and movement patterns.

If the amoebae are successfully cultured and identified, they can be subcultured into fresh batches of BM4020 Page's Saline (Amoeba Saline) or transferred to Non-Nutrient Agar for further study or experimentation. This allows for the maintenance and long-term cultivation of the amoebae.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>E. coli</i> (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Inhibited
<i>S. aureus</i> (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Inhibited

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 BM5500 Non-Nutrient Agar performance, samples were tested to assess inhibition of target bacteria when incubated at 37 ± 1°C aerobically for 18-24 hours. All samples showed no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that BM5500 Non-Nutrient Agar, meets performance criteria when used according to the instructions outlined above.

12. Limitations of the Medium

- 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 BM5500 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM5500 in the original bottle with the lid tightly closed at between 2 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 medium 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. Page, F. (1967) Taxonomic criteria for limax amoebae, with descriptions of 3 new species of Hartmannella and 3 of Vahlkampfia. The Journal of Protozoology, 14(3), pp. 499-521.

Version History*

001 10/06/24 - New Document Created

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



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TABLE OF APPLICABLE SYMBOLS					
REF Catalogue number	LOT Batch code	IVD In vitro Diagnostic Medical Device	Manufacturer	Use by	
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place	

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