

Instructions for Use

REF - BM4015

Buffered Nitrate Motility Medium

Bottled Media

1. Intended Use

BM4015 Buffered Nitrate Motility Medium is recommended for use in the confirmatory testing of *Clostridium perfringens* in water samples.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Beef extract	3.0
Peptone	5.0
Potassium nitrate	5.0
D-galactose	5.0
Glycerol	5.0
Disodium hydrogen phosphate	2.5
Agar	3.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This is a modification of the original Nitrate Reduction Broth which was generally used as one of a series of tests for the identification of *Enterobacteriaceae*. In addition to performing the test for nitrate reduction, this formulation also contains agar allowing detection of motility. The medium is used to confirm the presence of *C. perfringens* in water samples.

4. Principle

The Nitrate Reduction Test detects the presence of the enzyme nitrate reductase which, in the presence of an appropriate electron donor, reduces nitrate to nitrite. Following incubation, sulphanilic acid and α -naphthylamine dissolved in an acetic acid solution are added and a positive reaction is indicated by the formation of a red colour. Peptone and beef extract provide nitrogen and other essential nutrients. Galactose and glycerol are fermentable sources of carbon. Phosphate buffer is present to help maintain pH and agar produces the semi-solid gel required to detect motility.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Straw semi-solid gel	7.3 $\pm$ 0.2

6. Materials Provided

BM4015 Buffered Nitrate Motility Medium is available in the formats detailed below. Each bottle is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
BM4015-M002-10	50 x 10ml in Thin Wall Universals
BM4015-M002-20	50 x 20ml in Thin Wall Universals

7. Materials Needed but not Provided

Nitrate Reagents A and B required to conduct the nitrate reductase test.

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

8. Specimens

BM4015 Buffered Nitrate Motility Medium is suitable for the testing of the following specimens:

- The inoculum should be from a pure, overnight culture grown on a solid medium and not from a broth suspension

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

Stab-inoculate the medium with a loop or needle loaded with bacteria taken from a colony and incubate at 37 $\pm$ 1°C for 18-24 hours. After incubation, motility is apparent by diffuse growth away from the line of inoculation.

- Motility: Examine the tube for diffuse growth away from the stab line, indicating motility.

- Nitrate Reduction: Add 1 drop of Nitrate Reagent A (0.8% sulphanilic acid in 30% acetic acid solution) followed by one drop of Nitrate Reagent B (0.6% N,N-dimethyl-1-naphthylamine in 30% acetic acid solution) to the medium. A positive reaction will be indicated by the development of a red colour within 5 minutes of the addition of the reagents.

Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates from subcultures. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>C. perfringens</i> (NCTC 8237)	37 ± 1°C for 18-24 hours	Motile = -ve (no growth away from the stab). Nitrate reduction = +ve
<i>C. bifermentans</i> NCTC 506	37 ± 1°C for 18-24 hours	Motile = +ve (Medium should be turbid compared to uninoculated medium). Nitrate reduction = -ve
<i>E. coli</i> (NCTC 12241)	37 ± 1°C for 18-24 hours	Motile = +ve (Medium should be turbid compared to uninoculated medium). Nitrate reduction = +ve

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 the performance of BM4015 Buffered Nitrate Motility Medium, samples were tested to assess motility and the presence of nitrate reductase (nitrite) when incubated at 37 ± 1°C for 18-24 hours. All samples produced expected results from motility and nitrate reductase tests (see QC table above) of the required organisms: *Clostridium perfringens* (NCTC 8237), *Clostridium bifermentans* (NCTC 506) and *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that BM4015 Buffered Nitrate Motility Medium, 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 BM4015 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM4015 in the original bottle with the lid tightly closed at between 2 and 8°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

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Version History*

001 24/10/23 - 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

IFU/BM4015 REV. 001

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