

Instructions for Use

REF - BM1328

Acid Buffer (Double Strength)

Bottled Media

1. Intended Use

BM1328 Acid Buffer (Double Strength) is used to aid in the selective isolation of *Legionella* spp.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
0.4M Hydrochloric acid	135.0
0.4 M Potassium chloride	865.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Legionella spp. are aerobic, motile, nutritionally fastidious, gram-negative rods that require special growth factors in culture media to grow. Transmission occurs through aerosolization or aspiration of contaminated water.

Legionellosis refers to two distinct clinical syndromes: Legionnaires' disease, which most often presents as severe pneumonia, and Pontiac fever, which is an acute, febrile, self-limiting, viral-like illness. Over fifty species of *Legionella* have been identified, however, *Legionella pneumophila* remains the most prevalent etiological agent of Legionnaires' disease.

Legionellae are more resistant to lower pH and brief exposure to higher temperatures than many other freshwater bacteria. For some water samples with high levels of bacteria, it is necessary to use a selective procedure to reduce the number of non-*Legionella* bacteria before culture. Acid treatment of samples with acid buffer is routinely used for this purpose and to aid antigen extraction according to ISO 11731:2017 ⁽¹⁾.

4. Principle

Hydrochloric acid and potassium chloride in water form a pH buffer at low pH values. This allows the production of a relatively stable, low-pH aqueous solution that is suitable for the inactivation or destruction of most freshwater bacteria other than the acid tolerant legionellae. The product is prepared at twice the required concentration (double strength) to allow for the addition of equal amounts of the water sample under test.

5. Physical Characteristics

	<u>Appearance and Colour</u>	<u>pH</u>
Medium	Colourless liquid	2.2 ± 0.2

6. Materials Provided

BM1328 Acid Buffer (Double Strength) can be provided in the formats detailed below. Each bottle is labelled with (abbreviated) product name, product code, lot number and expiry date.

<u>Product Code</u>	<u>Product Format</u>
BM1328-A125-100	10 x 100ml in 125ml Sirop Bottles
BM1328-R125-100	10 x 100ml in 125ml Sirop Bottles

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

As BM1328 Acid Buffer (Double Strength) is suitable for the testing of the following specimens:

- Water/Environmental: Water from heating systems, cooling towers and air conditioning systems.

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

Based on sample type and information provided, inoculate plated media directly with the sample, or subculture onto plated media after treatment described in national guidelines.

Water/Environmental Samples ⁽¹⁾:

According to ISO 11731:2017, the method required for enumerating *Legionella* spp. is determined by the assumed sample content. Non-selective media such as Legionella BCYE Agar is recommended when processing samples that are presumed to have higher concentrations of *Legionella* species in combination with lower numbers of interfering microorganisms. In contrast,

selective media such as Legionella GVPC Agar is recommended when processing samples that are presumed to have lower concentrations of *Legionella* species in combination with higher numbers of interfering microorganisms. These samples are processed as required by the local guidelines and incubated until suspected *Legionella* colonies are found.

- Process the water/environmental sample as required by the local guidelines.
- Inoculate samples onto non-selective medium Legionella BCYE Agar, and onto selective media Legionella GVPC Agar if required.
- Incubate the plates $36 \pm 2^\circ\text{C}$ in 5% CO_2 and high humidity for up to 7 days (further incubation up to 10 days may be required to present growth of wild *Legionella* strains).

Colonies suspected of being *Legionella* spp. are subcultured to Legionella BCYE Agar and Legionella BCYE Cysteine Free Agar.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates ⁽²⁾. Refer to relevant local guidelines.

10. Quality Control

Tested for sterility only. Incubate the required number of samples at $37 \pm 1^\circ\text{C}$ for 72 hours and a second set at $20 \pm 5^\circ\text{C}$ for 72 hours. After 48 hours, subculture 10µl of one sterility broth (one from the $20 \pm 5^\circ\text{C}$ set and one from the $37 \pm 1^\circ\text{C}$ set) onto E&O PP0120 Columbia Blood Agar and incubate at the respective temperatures for 18-24 hours.

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 sterility of BM1328 Acid Buffer (Double Strength), samples were incubated at $37 \pm 1^\circ\text{C}$ for 72 hours and $20 \pm 5^\circ\text{C}$ for 72 hours and subcultured onto E&O PP0120 Columbia Blood Agar before being incubated for a further $37 \pm 1^\circ\text{C}$ for 18-24 hours and $20 \pm 5^\circ\text{C}$ for 18-24 hours. No growth was observed, and samples were concluded to be sterile. Therefore, BM1328 Acid Buffer (Double Strength), meets criteria to be used in microbial testing.

12. Limitations of the Media

As BM1328 Acid Buffer (Double Strength) is not to be used independently and is instead designed to be used as a reagent, limitations will vary depending on the procedure being followed with regards to the medium being used.

13. Precautions and Warnings

Refer to BM1328 Material Safety Data Sheet. Conduct full COSHH assessment for the local laboratory.

Causes serious eye damage and skin burns. Do not breath mist or vapour. Wear protective gloves/protective clothing/eye protection/face protection as determined by the local 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.

14. Storage conditions and Shelf life

Store BM1328 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 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. International Organization for Standardization (2017) 11731:2017 Water quality – Enumeration of Legionella. Geneva, ISO.
2. Edelstein, P. (1983) Comparative Study of Selective Media for Isolation of Legionella pneumophila from Potable Water. Journal Of Clinical Microbiology, 18(3): 751-751.

Version History*

001 23/10/23 - 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/BM1328 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