

Instructions for Use

REF - KM0093

Brucella Medium Base

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0093 Brucella Medium Base is used to make Brucella Medium, a general-purpose, non-selective medium for the cultivation of *Brucella* species and other fastidious, slow growing, obligately anaerobic microorganisms.

2. Composition*

Ingredient	g/L
Peptones	22
Sodium chloride	5
Glucose	1
Sodium bisulfite	0.1
Agar	14.9

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Brucella Medium Base is based on the APHA formulation for Albimi broth ⁽¹⁾. The product is recommended by APHA for the isolation of fastidious, anaerobic bacteria from food and dairy products ⁽¹⁾. Summanen *et al.* initially added Vitamin K to the formula ⁽²⁾ before Weinstein and Onderdonk further modified the medium with the addition of hemin ⁽³⁾. The incorporation of these additives, as well as sheep or horse blood ⁽⁴⁾, has made this medium appropriate for the cultivation of many anaerobes. This medium has also been suggested for use in anaerobe MIC testing methods.

4. Principle

The peptones function as a source of nitrogen and other essential nutrients. Glucose is a fermentable sugar and sodium chloride maintains the osmotic balance of the medium. Bisulphite is added as a reducing agent. Other ingredient may be added to support the growth of fastidious organisms (see Section 3).

5. Preparation Instructions

Suspend 43.0g/L of dried culture media in deionised/purified water. Add the required amount of haemin and mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling to 45-50°C, after which required amounts of vitamin K and defibrinated blood (Horse: E&O DHB; Sheep: E&O DSC) may be added as required.

Gently mix the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Straw firm gel
pH	N/A	7.0 ± 0.2

7. Materials Provided

KM0093 is available in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0093-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0093-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0093-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Quantities of haemin, vitamin K and defibrinated blood (Horse: E&O DHB; Sheep: E&O DSC) as required by the recipe being followed.

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

9. Specimens

KM0093 Brucella Medium Base when prepared as outlined in Section 5, is suitable for the testing of the following specimens:

- Clinical Specimens: may include (but not limited to) wound swabs, biopsy material, pus, faeces, and other samples from suspected anaerobic infections.

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. Refer to relevant guidelines.

10. Test Procedures and Interpretation of results

Follow local, national, or international guidelines.

Allow the plates to come to room temperature.

Clinical specimens:

- Process the clinical specimen as required by relevant guidelines.
- Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates anaerobically at $37 \pm 1^\circ\text{C}$ for 24-48 hours.

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

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>C. perfringens</i> (NCTC 8237)	$37 \pm 1^\circ\text{C}$ anaerobically for 24-48 hours	Growth: Grey colonies
<i>B. fragilis</i> (NCTC 9343)	$37 \pm 1^\circ\text{C}$ anaerobically for 24-48 hours	Growth: Grey 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).

12. Performance

To fully verify KM0093 Brucella Medium Base performance, dehydrated culture media samples were used to prepare Brucella Medium (with haemin, vitamin K and defibrinated sheep blood) and tested to assess colony morphology and recovery level (where an acceptable range is $\geq 70\%$ and $\leq 120\%$) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and incubated at $37 \pm 1^\circ\text{C}$ anaerobically for 24-48 hours. All samples of prepared media showed good recovery and the correct morphology of the required test organisms: *Clostridium perfringens* (NCTC 8237) and *Bacteroides fragilis* (NCTC 9343). Therefore, it can be concluded that KM0093 Brucella Medium Base meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

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

14. 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 KM0093 Material Safety Data Sheet.

15. Storage Conditions and Shelf Life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture medium does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Hausler, W. (ed.) (1976) Standard methods for the examination of dairy products, 14th ed. Washington DC: APHA.
2. Summanen, P., Baron, E., Citron, D., Strong, C., Wexler, H. and Finegold, S. (1993) Wadsworth Anaerobic Bacteriology Manual. 5th ed. Star Publishing Co., Belmont, CA.
3. Weinstein, W., Onderdonk, A., Bartlett, J., and Gorbach, S. (1974) Experimental intra-abdominal abscesses in rats: development of an experimental model. Infection and immunity, 10(6), pp.1250-1255.

4. Islam, M., Islam, M., Rahman, M., Islam, K., Islam, M., Kamal, M. and Islam, M. (2023) Presence of *Brucella* spp. in milk and dairy products: a comprehensive review and its perspectives. Journal of Food Quality, 2023(1), 2932883.

Version History*

- 001 25/09/23 - New Document Created
- 002 20/09/24 - Updated to allow for the potential use of horse blood

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



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TABLE OF APPLICABLE SYMBOLS		IFU/KM0093 REV. 002			
REF o 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	