PRODUCT SPECIFICATION SHEET

Brucella Agar Base w/ Hemin and Vitamin K (DM633)

Intended Use
Brucella Agar Base w/ Hemin and Vitamin K (DM633) is recommended for cultivation of Brucella species and with addition of blood may be used for isolation and subculture of anaerobes.

Product Summary and Explanation
Brucella is a genus of Gram-negative bacteria and they are normal flora of the genital and urinary tracts of many animals including goats, pigs, cows and dogs. *Brucella* is the cause of brucellosis, which is a zoonosis transmitted by ingesting contaminated food (such as unpasteurized milk products), direct contact with an infected animal, or inhalation of aerosols; the disease is particularly common among abattoir workers.[1] Brucellosis in humans has a variable incubation period, an insidious or abrupt onset and no pathognomic symptoms or signs.
Brucella Agar with Hemin and Vitamin K is a modification of Brucella Agar that has been supplemented with hemin and vitamin K1 to support the growth of fastidious anaerobes, especially *Bacteroides, Prevotella,* and *Porphyromonas* when incubated anaerobically.[2-4] This is a highly enriched medium, which can be used for the isolation of *Brucella* and other anaerobic bacteria.[5,6]

Principles of the Procedure
Brucella Agar Base w/ Hemin and Vitamin K contains casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract which are sources of carbon, nitrogen and essential growth nutrients including B-complex vitamins. Dextrose serves as a source of carbon and energy. Addition of blood provides nutrients and helps to differentiate hemolytic organisms.[5,6] Hemin and vitamin K1 have been shown to be necessary for supporting the growth of certain strict anaerobes like *Bacteroides* species and gram-positive spore bearers like *Clostridium* species.[7] Sodium bisulfite lowers the redox potential to a range suitable for strict anaerobes.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Peptic digest of animal tissue	10.00
Yeast extract	2.00
Dextrose	1.00
Sodium chloride	5.00
Sodium bisulphite	0.10
Hemin	0.01
Vitamin K1	0.01
Agar	15.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

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Directions

1. Suspend 43.12 grams of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 lbs pressure for 15 minutes/validated cycle.
4. Cool to 50°C and aseptically add 5% v/v sterile defibrinated sheep blood.
5. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to tan homogeneous free flowing powder
Prepared Medium	Basal medium: Light amber coloured clear to slightly opalescent gel After addition of 5% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates
Reaction of 4.31% solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed in presence of 10% CO2 with added 5% v/v sterile defibrinated sheep blood, after an incubation at 35-37°C for 48 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Bacteroides fragilis ATCC 25285*	50 - 100	good-luxuriant
2.	*Clostridium perfringens ATCC 13124*	50 - 100	good-luxuriant

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

The specimen should be inoculated onto the plate (reduced earlier by placing under anaerobic conditions for 18- 24 hrs) as early as possible. Swab cultures are directly streaked. Non-swab cultures are inoculated using an inoculating loop. Incubation is carried out anaerobically at 35°C for at least 48 hours; however, negative results should be reported only after incubation for 7 days. Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

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1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Brucella Agar Base w/ Hemin and Vitamin K
Product Code : DM633
Available Pack sizes : 500gm

References

1. Baron E. J., Finegold S. M., (Eds.), 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.
2. Sutter V. L.,Citron D. M. and Finegold S. M., 1985, Wadsworth Anaerobic Bacteriology Manual, 4th Ed.,Star Publishing Co.,Belmont, Ca.
3. Onderdonk A. B., Weinstein W. M., Sullivan N. M. and Bartlett J. G., 1974, Infect. Immun., 10:1256.
4. Weinstein W. M., Onderdonk A. B., Bartlett J. G. and Gorbach S. L., 1974, Infect. Immun., 10:1250.
5. Zennette, Balows, Hausler and Shadomy, (Eds.), 1985, Manual of Clinical Microbiology, 4th Ed., ASM, Washington, D.C.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
7. Gibbons and MacDonald, 1960, J. Bacteriol., 80:164.

Further Information

For further information please contact your local MICROMASTER Representative.

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