



PRODUCT SPECIFICATION SHEET

Brucella Agar Base (DM047)

Intended Use

Brucella agar is used for selective isolation and cultivation of Brucella or Campylobacter species from clinical and nonclinical specimen.

Product Summary and Explanation

The slow growth of Brucella species, combined with their requirement for highly nutritious media requires that selective agents must be incorporated to prevent overgrowth of contaminant organisms from milk or veterinary tissues. Brucella Agar was developed for the cultivation of Brucella species from diagnostic specimens, such as blood, and from foods and other potentially contaminated material. Brucella Agar is prepared according to the APHA formula for Albimi Broth.⁽¹⁾ Brucella Agar is a general purpose medium for the cultivation of *Brucella* spp. and fastidious microorganisms including *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Neisseria meningitides*.⁽²⁾ With the addition of blood, Brucella Agar is used to determine bacterial hemolytic reactions.⁽²⁾ Brucella Agar can be used as a base for the isolation of *Campylobacter* spp.⁽²⁾ Brucellosis is a zoonotic disease with a domestic-animal reservoir.⁽³⁾ Transmission by milk, milk products, meat, and direct contact with infected animals are the usual routes of exposure.⁽³⁾ Brucella Agar is a modified medium formulated to support luxuriant growth of fastidious bacteria like *Brucella*, streptococci, pneumococci, *Listeria*, *Neisseria meningitides* and *Haemophilus influenzae*.⁽⁴⁾ Brucella Agar is also recommended by APHA for isolation of *Brucella* species from foods.⁽⁵⁾

Principles of the Procedure

Brucella Agar supports the growth of fastidious microorganisms due to their content of casein enzymic hydrolysate and peptic digest of animal tissue and yeast extract. The peptones supply organic nitrogen. The yeast extract is a potent source of the B-complex vitamins. Dextrose is utilized as an energy source. Sodium bisulfite is a reducing agent, and sodium chloride maintains the osmotic equilibrium. Agar is the solidifying agent in Brucella Agar. The medium can also be enriched with 5% v/v sterile defibrinated horse blood which supplies both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*. For selective isolation of *Brucella* species antibiotic mixtures in the form of freeze dried supplements are incorporated into the base.^(6,7,8)

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
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.
3. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.





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4. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions

1. Suspend 21.55 grams of the medium in 500ml of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C and aseptically add sterile 5% v/v inactivated Horse Serum (MS084, inactivated by heating at 56°C for 30 minutes) and rehydrated contents of one vial of Brucella Selective Supplement (MS043).

For *Campylobacter*:

1. Add rehydrated contents of 1 vial of Campylobacter Supplement-I (Blaser-Wang) (MS004) or Campylobacter Supplement-II (Butzler) (MS005) or Campylobacter Supplement-III (Skirrow) (MS007) and 5-7% defibrinated sheep blood to 500 ml sterile medium.
2. For growth enhancement add rehydrated contents of 1 vial of Campylobacter Growth Supplement (MS008). Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous, free flowing powder
Prepared Medium	Yellow coloured, clear to slightly opalescent 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 after an incubation at 35-37°C for 24-72 hours in presence of 10% CO₂ with added sterile 5% v/v inactivated horse serum (MS084) and Brucella Selective Supplement (MS043).

Sr.no	Organisms	Growth
1.	<i>Brucella melitensis</i> ATCC4309	good-luxuriant
2.	<i>Brucella suis</i> ATCC 4314	good-luxuriant
3.	<i>Escherichia coli</i> ATCC 25922	inhibited
4.	<i>Staphylococcus aureus</i> ATCC 25923	inhibited

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

Test Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Since many pathogens require carbon dioxide on primary isolation, incubate plates at 35 ± 2°C for 24-72 hours in anaerobic atmosphere supplemented with carbon dioxide.
3. Swab specimens can be directly streaked on the plate. Liquid specimens can be inoculated by means of an inoculation loop.
4. loop.
5. When non-selective medium is required, Brucella Broth Base may be employed with the addition of serum only (i.e. without antibiotics).
6. For direct culture of Brucella species from milk transfer the samples to sterile tubes and hold overnight at 40°C.
7. Withdraw an aliquot of gravity cream with a spiral wire and spread over a plate of supplemented agar with a bent sterile glass rod.
8. Incubate the plates at 35°C in an atmosphere containing 10±20% (v/v) carbon dioxide and examine every two days for ten days.





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9. *Brucella* colonies appear as 1±2mm diameter convex colonies with round entire edges, and may be identified by slide agglutination.

Results

1. After incubation, most plates will show an area of confluent growth.
2. The streaking procedure is, in effect, a "dilution" technique, hence diminishing numbers of microorganisms are deposited on the streaked areas.
3. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen.
4. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

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

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Product Name : Brucella Agar Base

Product Code : DM047

Available Pack sizes : 100gm / 500gm

References

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Further Information

For further information please contact your local MICROMASTER Representative.



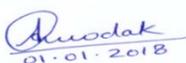


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