



PRODUCT SPECIFICATION SHEET

Bushnell Haas Broth (DM051)

Intended Use

Bushnell Haas Broth (DM051) is recommended for examining fuels for microbial contamination and for studying hydrocarbon deterioration by microorganisms.

Product Summary and Explanation

Bushnell Haas Broth is prepared as per the formula described by Bushnell and Haas⁽¹⁾ and recommended for the microbiological examination of fuels by the SIM Committee on microbiological deteriorations of fuels.⁽²⁾ These media contain all nutrients except carbon source, necessary for the growth of bacteria. This medium is used to evaluate the ability of microorganisms to decompose hydrocarbons. It is formulated without a carbon source which allows for the addition of alternative hydrocarbons such as kerosene, light and heavy mineral oils, paraffin wax and gasoline. For liquid hydrocarbon the hydrocarbon is layered on the surface of inoculated agar. For testing volatile hydrocarbons such as gasoline the Petri-plates containing the medium are inverted and the hydrocarbon is poured into the lid. The medium was used to enumerate total heterotrophs and hydrocarbon degradation by microorganisms during bioremediation of Prince William Sound following the Exxon *Valdez* oil spill.^(3, 4)

Principles of the Procedure

Bushnell Haas Broth contains magnesium sulphate, calcium chloride and ferric chloride which provide trace elements necessary for bacterial growth. Ammonium nitrate is a nitrogen source, while monopotassium phosphate and potassium phosphate buffers the medium.

Formula / Liter

| Ingredients | Gms / Liter |
|--|-------------|
| Magnesium sulphate | 0.20 |
| Calcium chloride | 0.02 |
| Monopotassium phosphate | 1.00 |
| Dipotassium phosphate | 1.00 |
| Ammonium nitrate | 1.00 |
| Ferric chloride | 0.05 |
| 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.

Directions

1. Suspend 3.27 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense desired amounts in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. A white precipitate prior to sterilization becoming yellow to orange after sterilization is normal.





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Quality Control Specifications

| | |
|-----------------------------------|--|
| Dehydrated Appearance | White to cream homogeneous free flowing powder |
| Prepared Medium | Colourless coloured clear to slightly opalescent solution in tubes |
| Reaction of 0.33% Solution | pH : 7.0 ± 0.2 at 25°C |
| Gel Strength | Not Applicable |

Expected Cultural Response: Cultural characteristics observed after an incubation at 25-30°C within 1 week.

| Sr. No. | Organisms | Results to be achieved | | |
|---------|--|------------------------|----------------|--------------------|
| | | Inoculum (CFU) | Growth (Plain) | Growth w/ Minerals |
| 1. | <i>Pseudomonas aeruginosa</i> ATCC 27853 | 50 -100 | Poor | good-luxuriant |
| 2. | <i>Pseudomonas aeruginosa</i> ATCC 9027 | 50 -100 | Poor | good-luxuriant |

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

Test Procedure

1. Inoculate the collected sample directly into the broth.
2. Overlay the broth with a sterile hydrocarbon source.
3. Incubate aerobically at 25-30°C.
4. Examine tubes daily for growth for up to one week.

Results

Organisms that are capable of degrading hydrocarbons should show growth in the Bushnell-Haas Broth when supplemented with a hydrocarbon source.

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

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 : Bushnell Haas Broth

Product Code : DM051

Available Pack sizes : 100gm/ 500gm

References

1. Bushnell and Haas. 1941. J. Bacteriol. 4:653.





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2. Allred, DeGray, Edwards, Hedrick, Klemme, Rogers, Wulf and Hodge. 1963. Proposed procedures for microbiological examination of fuels. SIM Special Publications, No. 1. Merck, Sharp & Dohme Research Laboratories, Rahway, N.J.
3. Bragg, Roffall and McMillen. 1990. Column flow studies of bioremediation in Prince William Sound. Exxon Production Research Co., Houston, Tex.
4. Brown and Braddock. 1990. Appl. Environ. Microbiol. 56:3895.

Further Information

For further information please contact your local MICROMASTER Representative.



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| | Checked By | Approved By |
|---|---|---|
|  |  |  |
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