# Bushnell-Haas Broth

## Intended Use

Bushnell-Haas Broth is used for studying microbial utilization of hydrocarbons.

## Summary and Explanation

Bushnell-Haas Broth (Bushnell-Haas marine salts broth), prepared according to the formula described by Bushnell and Haas[1], 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.

Bushnell-Haas Broth was recommended for the microbiological examination of fuels by the Society for Industrial Microbiology (SIM) Committee on Microbiological Deterioration of Fuels.[2] 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

Magnesium sulfate, calcium chloride and ferric chloride provide trace elements necessary for bacterial growth. Potassium nitrate is a nitrogen source, while monopotassium phosphate and diammonium hydrogen phosphate provide buffering capability.

## User Quality Control

### *Identity Specifications*

**Difco™ Bushnell-Haas Broth**

| | |
|---|---|
| Dehydrated Appearance: | Beige with pink tint, free-flowing, homogeneous (may contain small dark particles). |
| Solution: | 0.327% solution, partially soluble in purified water, white precipitate remains. Solution, after autoclaving, is colorless to very light amber, clear supernatant over yellow-orange precipitate. |
| Prepared Appearance: | Colorless to very light amber, clear supernatant over yellow-orange precipitate. |
| Reaction of 0.327% Solution at 25°C: | pH 7.0 ± 0.2 |

### *Cultural Response*

**Difco™ Bushnell-Haas Broth**

Prepare the medium per label directions. Inoculate in duplicate with the test organisms. Add sterile mineral oil (the hydrocarbon source) to one set. Incubate at 25-30°C for up to 1 week.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY WITHOUT HYDROCARBON | RECOVERY WITH HYDROCARBON |
|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 9027 | $10^2$-$10^3$ | None to poor | Good |
| *Pseudomonas aeruginosa* | 10145 | $10^2$-$10^3$ | None to poor | Good |
| *Pseudomonas aeruginosa* | 14207 | $10^2$-$10^3$ | None to poor | Good |
| *Pseudomonas aeruginosa* | 27853 | $10^2$-$10^3$ | None to poor | Good |

## Formula

**Difco™ Bushnell-Haas Broth**

| Approximate Formula* Per Liter | | |
|---|---|---|
| Magnesium Sulfate | 0.2 | g |
| Calcium Chloride | 0.02 | g |
| Monopotassium Phosphate | 1.0 | g |
| Diammonium Hydrogen Phosphate | 1.0 | g |
| Potassium Nitrate | 1.0 | g |
| Ferric Chloride | 0.05 | g |

**Adjusted and/or supplemented as required to meet performance criteria.*

## Directions for Preparation from Dehydrated Product

1. Suspend 3.27 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: A precipitate, white prior to autoclaving becoming yellow to orange after autoclaving, is normal.

## 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.

## Expected Results

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

## References


1. Bushnell and Haas. 1941. J. Bacteriol. *41*:653.
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.


## Availability

**Difco™ Bushnell-Haas Broth**

Cat. No. 257820 Dehydrated – 500 g