TM 559 – BUSHNELL HAAS AGAR

INTENDED USE
For examination of fuels for microbial contamination and hydrocarbon deterioration by bacteria.

PRODUCT SUMMARY AND EXPLANATION
Bushnell Haas Agar is prepared as per the formula of Bushnell and Haas and recommended for the microbiological examination of fuels by the SIM Committee on microbiological deteriorations of fuels. These media contain all nutrients except carbon source, necessary for the growth of bacteria. Only those bacteria that are able to decompose hydrocarbon will grow in these media. Specific carbon source i.e. hydrocarbon can be added to this medium and their utilization by different microorganisms can be studied. These bacteria can decompose a variety of hydrocarbons like kerosene, mineral oil, 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.

COMPOSITION

Ingredients	Gms / Ltr
Magnesium sulphate	0.200
Calcium chloride anhydrous	0.200
Potassium dihydrogen phosphate	1.000
Dipotassium hydrogen phosphate	1.000
Ammonium nitrate	1.000
Ferric chloride	0.050
Agar	20.000

PRINCIPLE
Magnesium sulphate, calcium chloride and ferric chloride provide trace elements. Ammonium nitrate is a nitrogen source while monopotassium phosphate and potassium phosphate buffers the medium.

INSTRUCTION FOR USE
- Dissolve 23.27 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.
- A white precipitate prior to sterilization becoming yellow to orange after sterilization is normal.

QUALITY CONTROL SPECIFICATIONS
Appearance of Powder : White to cream homogeneous free flowing powder.
Appearance of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.0±0.2

INTERPRETATION
Cultural characteristics observed after incubation.

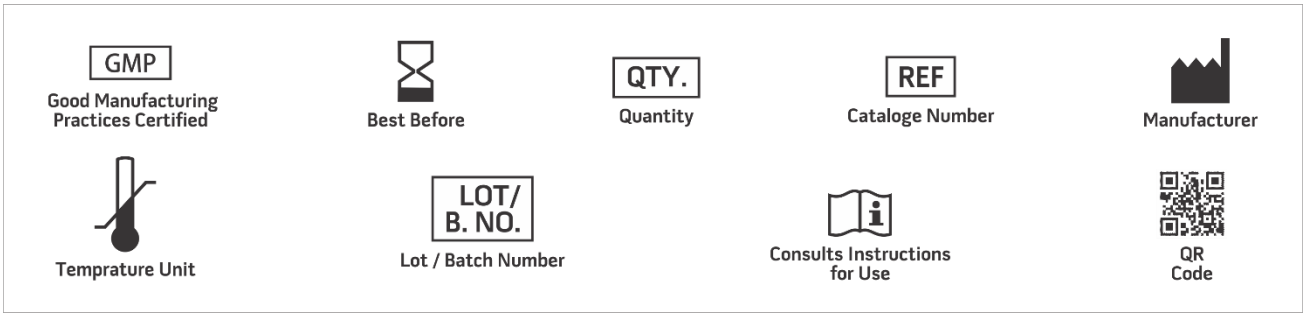
Microorganism	ATCC	Inoculum (CFU/ml)	Growth (plain)	Recovery (plain)	Growth w/minerals	Recovery (plain)	Incubation Temperature	Incubation Period
Pseudomonas aeruginosa	27853	50-100	Poor	10-20%	Good-luxuriant	>=50%	25-30°C	Within 1 week
Pseudomonas aeruginosa	9027	50-100	Poor	10-20%	Good-luxuriant	>=50%	25-30°C	Within 1 week

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
1. 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.
2. Bushnell and Haas, 1941, J. Bacteriol., 41:653.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
Revision: 08 Nov., 2019