

## Technical Data Sheet

### GranuCult® prime Brain Heart Infusion (BHI) agar acc. FDA-BAM

Ordering number: 1.03870.0500

For the cultivation and maintenance of fastidious microorganisms from food and animal feed, water and other samples.

This culture medium complies with the specifications given by APHA Part 9230C, ASTM D5259, FDA-BAM Medium M24 (Medium 1) and USDA-FSIS.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

#### Mode of Action

This culture medium can be used as a general-purpose medium, it contains no inhibitors or dyes and a broad spectrum of bacteria and fungi are able to grow on it. The highly nutritious substrate prepared from extracts of brain and heart, and peptones provide nitrogen, vitamins, amino acids and carbon sources. Glucose is an additional carbon source whilst disodium phosphate acts as a buffer. Sodium chloride maintains the osmotic balance and agar is the solidifying agent.

Brain Heart Infusion (BHI) agar poured in slants is used for maintenance of pure cultures of microorganisms.

Brain Heart Infusion (BHI) agar is less suited for identifying hemolytic forms when blood has been added due to its glucose content.

According to APHA Compendium of Methods for microbiological examination of foods, BHI agar may be supplemented with vancomycin for the selective screening of vancomycin-resistant enterococci (VRE) from food samples.

## Typical Composition

Specified by FDA-BAM M24 (Medium 1), USDA-FSIS		Specified by APHA Part 9230C		GranuCult® prime Brain Heart Infusion (BHI) agar acc. FDA-BAM	
Calf brain, infusion from 200 g		Calf brain, infusion from 200 g		Nutrient Substrate (extracts of brain and heart, and peptones)	27.5 g/l
Beef heart, infusion from 250 g		Beef heart, infusion from 250 g			
Proteose peptone	10.0 g/l	Proteose peptone	10.0 g/l		
Dextrose	2.0 g/l	Glucose	2.0 g/l	D(+)-Glucose*	2.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l
Na <sub>2</sub> HPO <sub>4</sub>	2.5 g/l	Na <sub>2</sub> HPO <sub>4</sub>	2.5 g/l	Na <sub>2</sub> HPO <sub>4</sub>	2.5 g/l
Agar	15.0 g/l	Agar	15.0 g/l	Agar-agar**	15.0 g/l
Water	1000 ml	Water	1000 ml	Water	n/a
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

\* D(+)-Glucose is equivalent to the term Dextrose.

\*\*Agar-Agar is equivalent to other different terms of agar.

## Preparation

Dissolve 52.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Pour to plates or slants.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear to opalescent and yellowish-brown. The pH value at 25 °C is in the range of 7.3 - 7.7.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubate the inoculated medium at (34–38) °C for (24–48) hours aerobic. If required, incubate in a microaerophilic or anaerobic atmosphere. In the case of longer incubation times the plates must be protected against desiccation.

**Following the procedure given by APHA Part 9230C and ASTM D5259 for verification of *Enterococcus* and *Streptococcus* species**, select typical colonies for isolation and confirmation. Streak each of the selected colonies onto the surface of a BHI agar plate or slant and incubate at (35 ± 0.5) °C between (24 ± 2) and (48 ± 3) hours. Perform subsequent confirmation tests as given by APHA Part 9230C / ASTM D5259.

## Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According to McFaddin, self-prepared plates and tubes can be stored in the dark and protected against evaporation at (5 ± 3 °C) for up to six months.

## Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133.

Test method: Performance testing of solid culture media - Quantitative and qualitative methods

Quantitative method for solid media	
Test strain	Specification Recovery rate / growth
<i>Staphylococcus aureus</i> ATCC® 25923 [WDCM 00034]	≥ 70 %
<i>Listeria monocytogenes</i> ATCC® 13932 [WDCM 00021]	≥ 70 %
<i>Enterococcus faecalis</i> ATCC® 19433 [WDCM 00009]	≥ 70 %
<i>Escherichia coli</i> ATCC® 25922 [WDCM 00013]	≥ 70 %
<i>Candida albicans</i> ATCC® 10231 [WDCM 00054]	≥ 70 %
<i>Aspergillus brasiliensis</i> ATCC® 16404 [WDCM 00053]	weak to good growth

Incubation: 24 ± 2 hours at 35 ± 1 °C aerobic, *Aspergillus* up to 3 days at 35 ± 1 °C aerobic.  
Reference medium: Tryptic Soy agar.

A recovery rate of 70 % is equivalent to a productivity rate of 0.7.

Please refer to the actual batch related Certificate of Analysis.

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## Literature

APHA (2018): Part 9230: Fecal Enterococcus/Streptococcus groups. Part C: Membrane filter techniques. Standard Methods for the Examination of Water. 23<sup>rd</sup> ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

APHA (2015) Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5<sup>th</sup> ed. American Public Health Association, Washington, D.C.

ASTM American Society for Testing and Materials. Standard Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure. ASTM D5259:2019.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

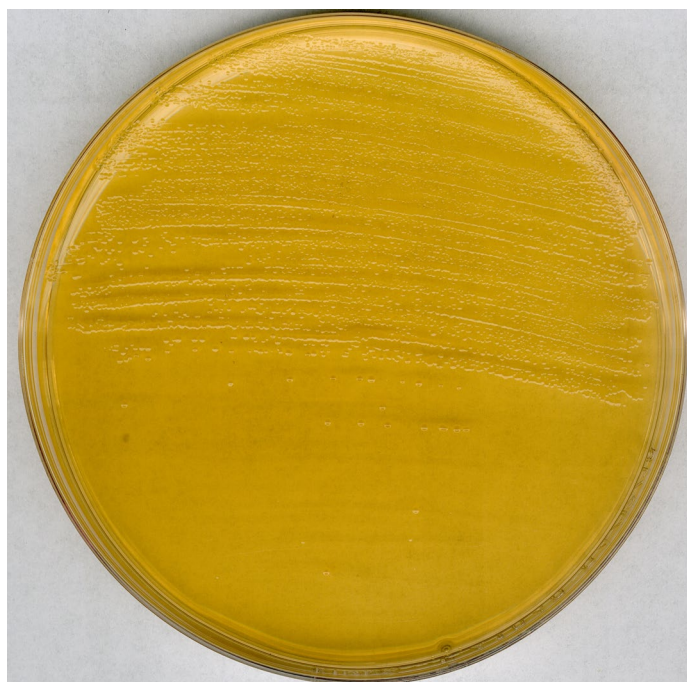
FDA-BAM (2017): Chapter No. 23: Microbiological methods for cosmetics. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M24: Brain Heart Infusion (BHI) Agar. Food and Drug Administration - Bacteriological Analytical Manual.

USDA-FSIS (2017): Microbiological Laboratory Guidebook (MLG) Appendix 1.09: Brain Heart Infusion (BHI) Agar. United States Department of Agriculture – Food Safety and Inspection Service.

Klein G. and Reuter G. (2012): Culture media for *Enterococci*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 155-173. Royal Society of Chemistry, Cambridge, UK.

McFaddin J.F. (1985): Brain Heart Infusion (BHI) Agar/Broth. In: Media for isolation – cultivation – identification – maintenance of medical bacteria. Volume I. pp. 92-95. Lippincott Williams and Wilkins, Baltimore, MD, USA.



*Clostridium perfringens* ATCC 10543 on Brain Heart Infusion agar

## Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime Brain Heart Infusion (BHI) agar acc. FDA-BAM	1.03870.2601	500 g

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