HHD BROTH

Dehydrated culture medium

1 - INTENDED USE

For the detection and differentiation of heterofermentative and homofermentative lactic acid bacteria.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Fructose	2.500 g
Potassium dihydrogen phosphate	2.500 g
Tryptic digest of casein	10.000 g
Soy peptone	1.500 g
Acid digest of casein	3.000 g
Yeast extract	1.000 g
Bromocresol green	0.066 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactic Acid Bacteria are widely distributed throughout nature and are involved not only in the production, but also in the spoilage of acid food products. The Lactic Acid Bacteria (LAB), although consisting of a number of diverse genera, are grouped as either homofermenters or heterofermenters on the basis of their metabolic properties: the heterofermentative LAB, which produce CO_2, lactic acid, acetic acid, ethanol, and mannitol from hexoses, and the homofermentative LAB, which produce primarily lactic acid from hexose.
HHD Broth, prepared according to the formula of McDonald et al.[1], allows differentiation of the two groups of bacteria on the basis of a different acidification of the substrate that contains a low amount of fructose (14mM). The addition of agar to HHD Broth allows differentiation by colony colour in a solid medium. HHD Agar is recommended by APHA[2] for enumeration of acid-producing microorganisms in fermented and acidified vegetables.
Bromocresol green is used as a pH indicator that differentiates the degree of acidification induced by both groups of bacteria. The indicator is yellow at pH values below 8.8 and blue at values above 5.6. The homofermentative bacteria produce 2 moles of lactic acid from fructose and grow on HHD Broth with a colour change of the indicator to green and blue-green sedimented cells at the bottom of the tube.
Heterofermentative bacteria induce less acidification of the substrate and grow on HHD Broth without significantly changing the colour of the medium, which remains blue with white sedimented cells.
If agar is added to HHD Broth, the homofermentative bacteria grow with blue-green colonies, the heterofermentative ones with colourless colonies.
McDonald *et al.*[1] recommend recording the colour of the cell sediment and colonies for the differentiation of the two bacterial groups rather than the colour change of the medium.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 20.6 g in 1000 mL of cold purified water and add 1g of Tween 80 (REF 42120502). Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.
For the preparation of HHD Agar, add 13 g/L of Agar Bios LL (REF 411030) and add 1g of Tween 80 (REF 42120502) to HHD Broth before sterilisation.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue, limpid
Final pH at 20-25 °C	7.0 ± 0.1

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
HHD Broth	Dehydrated medium	4015292	500 g (24.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, sterile Petri dishes, ancillary culture media and reagents.

8 – SPECIMENS

Food samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

9 - TEST PROCEDURE

HHD Broth

1. Inoculate the tubes with the sample suspension and/or its decimal dilutions or with pure cultures.
2. Incubate at 30°C for 3 days.

HHD Agar[2]

1. Samples are pour-plated with melted HHD Agar and after solidification are overlayed with additional HHD Agar.
2. Plates are incubated at 30 °C for 72 ± 3 hours.

10 - READING AND INTERPRETATION

HHD Broth: the homofermentative bacteria grow with a green/blue-green cell sediment at the bottom of the tube whereas the heterofermentative bacteria grow without significantly changing the colour of the medium, which remains blue with a white cell sediment.
HHD Agar: homofermentative organisms grow with blue to green colonies, whereas heterofermentative colonies remains white.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T	EXPECTED RESULTS
L. mesenteroides ATCC 14935	30°/ 72 hours	growth, the medium remains blue, white sedimented cells
L. plantarum ATCC 14917	30°/ 72 hours	growth, the medium turn to green, green sedimented cells

ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated HHD Broth is tested for productivity and differential properties by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 30°C for 72 hours and recording the highest dilution showing growth and the colour of sedimented cells in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following homofermentative strains: *L. acidophilus* ATCC 314, *L. plantarum* ATCC 14917, *B. bifidum* ATCC 11863, and with the following heterofermentative strains: *L. dulbrueckii* var *bulgaricus* DSM 20081, *L. mesenteroides* ATCC 14935. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1 and the colour of the sedimented cells shall be conform to specifications.

13 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

15 – REFERENCES

1. Mc Donald LC, Mc Feeters RF, Daeschel MA, Fleming HP. Differential medium for the enumeration of homofermentative and heterofermentative lactic acid bacteria. App Environ Microbiol 1987; 53:1382-1384.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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