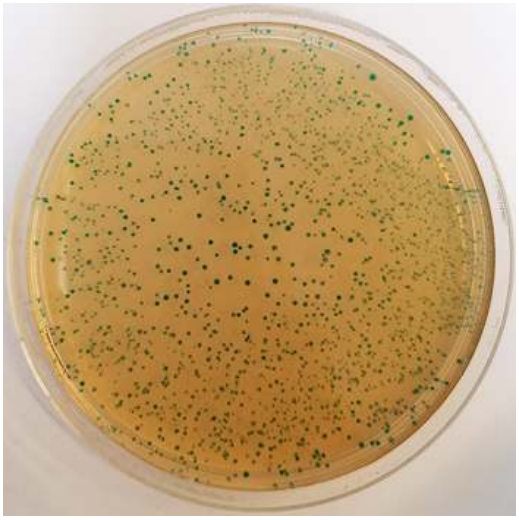
CHROMART

CHROMOGENIC LACTOBACILLUS ACIDOPHILUS AGAR (CLAA)

Dehydrated culture medium



L.acidophilus on CLAA

1 - INTENDED USE

Chromogenic medium for the enumeration and differentiation of *Lactobacillus acidophilus* in yogurt and acid milk.

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

Tryptone	10.00 g
Yeast Extract	5.00 g
Potassium dihydrogen phosphate	6.000 g
Di-ammonium citrate	2.000 g
Sodium acetate	15.00 g
Magnesium sulphate anhydrous	0.281 g
Ferrous sulphate	0.034 g
Manganous sulphate anhydrous	0.011 g
Glucose	20.00 g
Polysorbate 80	1.00 g
Agar	12.00 g
X-Glu^	0.02 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria
^ 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Yogurt-related milk products are increasingly being used as carriers of probiotic bacteria for their potential health benefits. To meet with a recommended level of ≥10^6 viable cells/g of a product, assessment of viability of probiotic bacteria in market preparations is crucial.[1]
Chromogenic Lactobacillus Acidophilus Agar (CLAA) is a selective and chromogenic medium for the enumeration and differentiation of *L.acidophilus* in yogurt-related milk products such as yogurt and acid milk.[2]
CLAA medium contains two peptones and yeast extract as sources of nitrogen, carbon and vitamins, necessary for microbial growth. Dextrose provides carbon and is source of energy. Polysorbate 80 acts as surfactant and provides fatty acids required for the metabolism of lactobacilli. Ammonium citrate and sodium acetate inhibit the growth of streptococci, moulds, and other oral microbial flora and restrict *Proteus* swarming. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate, ferrous sulphate and manganous sulphate are sources of inorganic ions for the optimal growth of lactobacilli. The detection principle is based on the specific visualization of the β-D-glucosidase activity of *L.acidophilus* via a chromogenic reaction of 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (X-Glu) which is cleaved with the formation of blue-green colonies.[2]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 71.3 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes, cool to 45-50°C and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Lactobacillus Acidophilus Agar (CLAA)	Dehydrated medium	4015682 4015684	500 g (7 L) 5 kg (70 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile microbiological loops or needles, incubator and laboratory equipment as required, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

The specimens consist of yogurt-related milk products such as yogurt and acid milk. Refer to the applicable international Standards for samples collection and preparation.[3] The medium is not intended for microbiological examination of clinical specimens.

9 - TEST PROCEDURE

Prepare the sample suspension and further decimal dilutions with Maximum Recovery Diluent or another suitable diluent. Transfer by means of a sterile pipette 0.1 mL of the test sample if liquid or 0.1 mL of the initial suspension in the case of other products, to each of two agar plates containing the CLAA medium. Repeat the procedure for further decimal dilutions if necessary. Carefully spread the inoculum as quickly as possible over the surface of the agar plate. Allow the medium to absorb the sample. Invert the plates and incubate anaerobically at 35-37°C for 72 h ± 3 hours.

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10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Characteristic *L.acidophilus* colonies are blue-green (β-glucosidase positive).
β-glucosidase negative lactobacilli, such as *L.casei* and *L.delbrueckii* subs *bulgaricus,* grow with white colonies.
Enumerate the colonies showing the features of characteristic microorganisms on plates having between 10 and 300 colonies.

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, it is responsibility of the end-user to perform Quality Control testing 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 / ATM	EXPECTED RESULTS
L.acidophilus ATCC 314	35-37° / 70-74 H /AN	growth with blue-green colonies
L.delbrueckii subs *bulgaricus* DSM 20081	35-37° / 70-74 H /AN	growth with white colonies
L.casei ATCC 393	35-37° / 70-74 H /AN	growth with white colonies

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection: DSM: German Collection of Microorganisms and Cell Cultures

12 - LIMITATIONS OF THE METHOD

- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on CLAA medium.

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates 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 product 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 +2°C /+8°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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

15 - REFERENCES

1. Ashraf F., Shah NP. Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt--a review. Int J Food Microbiol. 2011 Oct 3;149(3):194-208.
2. Kneifel W, Pacher B. An X-Glu based agar medium for the selective enumeration of *Lactobacillus acidophilus* in yogurt-related milk products. International Dairy Journal 1993; 3:277
3. ISO 20128:2006 [IDF 192:2006]. Milk products -Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium - Colony-count technique at 37 °C.

TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First publication	2021/05

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

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