



DESOXYCHOLATE LACTOSE AGAR

Dehydrated culture medium

1 - INTENDED USE

Slightly selective medium for the isolation and differentiation of Gram-negative enteric bacteria and for the enumeration of coliforms from water, wastewater, milk and dairy products.

2 - COMPOSITION -TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.000 g
Lactose	10.000 g
Sodium chloride	5.000 g
Sodium citrate	2.000 g
Sodium deoxycholate	0.500 g
Neutral red	0.033 g
Agar	15.000 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

Desoxycholate Lactose Agar is prepared on the basis of a modification of the formulation described by Leifson¹ in 1935 by lowering the concentration of sodium deoxycholate. Desoxycholate Lactose Agar was recommended by APHA^{2,3} in pour plate procedures for isolation and enumeration of coliforms in milk, water and other specimens. The medium is no longer included in recent editions of these manuals. Peptocomplex provides nitrogen, carbon and trace elements for microbial growth; sodium chloride maintains the osmotic balance; the slight selectivity is due to the presence of sodium deoxycholate and sodium citrate which allow a good growth of Gram-negative bacteria and a partial inhibition of Gram-positive bacteria. Lactose is included into the medium as a fermentable carbohydrate, neutral red as a pH indicator: coliforms ferment lactose producing an acidification of the medium and exhibiting colonies with red colour, often surrounded by a pink-red opaque halo due to the precipitation of sodium deoxycholate. Bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and boil for 1 minute for dissolve the medium completely. Cool to 47-50 °C, mix well and pour into sterile Petri dishes. Do not sterilize in autoclave and do not overheat.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige to pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Desoxycholate Lactose Agar	Dehydrated medium	4013802	500 g (11.7 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Desoxycholate Lactose Agar is intended for the bacteriological processing of water, wastewater, milk, dairy products and other samples of sanitary significance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Enumeration of coliforms in water and dairy products:⁴

- Introduce 1-4 mL of sample (and/or decimal dilutions of the sample) into sterile Petri dishes.
- Add 10-20 mL of medium cooled to 44-47°C and mix well the medium with the inoculum.
- Allow the medium to solidify and pour a surface covering layer of 3-4 mL of uninoculated medium.
- Incubate aerobically at 35-37°C for 18-24 hours. If negative after 24 hours, re-incubate additional 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Gram negative bacteria grow with different characteristics depending on their ability to ferment lactose and to induce the pH indicator changes.

Lactose fermenting Gram-negative enteric bacteria (coliforms): pink or bright red lenticular colonies sometimes surrounded by a red-pink opaque zone (precipitation of sodium deoxycholate).

Lactose non fermenting Gram-negative enteric bacteria: colourless 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, 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 / ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 25922	35-37°C / 18-24 H / A	growth, red-violet colonies surrounded by a red-pink opaque zone
<i>S. Enteritidis</i> ATCC 13076	35-37°C / 18-24 H / A	growth, colourless colonies
<i>S. aureus</i> ATCC 25922	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; 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 Desoxycholate Lactose Agar is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by a quantitative poured plate method with the target strains *E. coli* ATCC 25922 and *E. aerogenes* ATCC 13048; the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 18-24 hours. The colonies are enumerated on Test Batch and on Reference Batch and the productivity ratio (Pr: CFU_{TB}/CFU_{RB}) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (pink-red colonies with red opaque halo) the results are considered acceptable and conform to the specifications.

Specificity is tested by semi-quantitative ecometric technique with *S. Enteritidis* ATCC 13076 and *S. flexneri* ATCC 12022. After incubation, both strains exhibit a good growth with colourless colonies.

Selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target Gram positive strains *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923. The growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Aerobic or facultative anaerobic Gram-negative bacteria other than *Enterobacteriaceae* (e.g. *Pseudomonas*, *Aeromonas*) may grow on the medium with colourless colonies.
- Desoxycholate Lactose Agar does not contain an indicator system for the production of hydrogen sulphide therefore *Salmonella* colonies do not have a black centre.
- At pH above 7.5 the medium loses some of its inhibitory properties towards Gram positive bacteria.^{1,4}
- With overheating there may be a decrease in the gelling power of the agar due to its hydrolysis by sodium citrate.^{1,4}
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

14 - PRECAUTIONS AND WARNINGS

- This product 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 culture medium 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 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.

15 - 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 for the validation of the shelf life of the finished products, according to the type (plates/bottles) and the storage method (temperature and packaging). According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C for up to one week.⁴










16 - REFERENCES

- Leifson E. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. *J Pathol Bacteriol* 1935; 40: 581-599.
- American Public Health Association. Standard methods for the examination of water and wastewater, 11th ed. 1960.
- American Public Health Association. Standard Methods for the Examination of Dairy Products. 14th ed. 1967
- MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.





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 2	Updated layout and content	2022/07

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

