ENDO AGAR

Dehydrated culture medium

1 - INTENDED USE

For the detection and differentiation of coliforms and other of Gram-negative enteric bacteria.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.0 g
Lactose	10.0 g
Dipotassium hydrogen phosphate	3.5 g
Sodium sulphite	2.5 g
Pararosanilin (basic fuchsin)	0.4 g
Agar	11.0 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

Endo Agar was originally devised by Endo[1] for the isolation of the typhoid bacillus. Many modifications of this medium have been done over the years. Endo Agar has been recommended as a slightly selective medium for confirmation of the presumptive test for members of the coliform group and has been an important culture medium for the microbiological examination of water and wastewater, dairy products and foods;[2-4] however, Standard Methods for the examination of these materials now recommend alternative formulations.
Endo Agar is today used for the differentiation of lactose fermenting and non-lactose fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms and, in some areas, for the isolation and differentiation of *Enterobacteriaceae.*
Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals. Dipotassium phosphate is used as buffering agent to control the pH in the medium. A partial inhibition of Gram-positive bacteria is achieved without the traditional use of bile salts but with the inclusion of the combination sodium sulphite/acid fuchsin. Sodium sulphite in the medium also has the function of decolourising acid fuchsin as it occurs in Schiff's reagent. Lactose-fermenting bacteria produce acetaldehyde from lactose which releases the fuchsin from the colourless fuchsin-sulphite compound and colours the colonies red; when the reaction is rapid and very intense (e.g in the case of *E. coli*), the fuchsin crystallises out and produces a metallic sheen to the colonies. Non-lactose fermenting organisms produce colourless colonies against the pink background of the medium.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well for resuspending the precipitate and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder with small dark particles
Solution appearance	pink-orange, opalescent with small dark particles
Prepared medium appearance	pink rose to tan rose trace orange, slightly opalescent with small dark particles
Final pH at 20-25 °C	7.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Endo Agar	Dehydrated medium	4014602	500 g (12.1 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Water, wastewater, food, environmental samples. Consult the appropriate references for sample collection, storage and preparation.

9 - TEST PROCEDURE

Streak the sample suspension or the growth obtained in a selective broth (e.g. Brilliant Green Bile Broth) on Endo Agar plate.
Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *E. coli* colonies are pink to red with golden metallic sheen
Typical coliforms other than *E. coli* colonies are pink to red.
Typical non-lactose fermenters colonies are colourless against the pink background of the medium

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
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with metallic sheen
S. Enteritidis ATCC 13076	37°C/24H-A	good growth, colourless colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

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

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Endo Agar are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity and specificity characteristics are tested by semi-quantitative ecometric technique with lactose fermenting strains (*E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736) and non-lactose fermenting strains (*P. vulgaris* ATCC 13315, S. Enteritidis NCTC 5188, *P. aeruginosa* ATCC 14207). After incubation at 37°C for 24 hours, the amount of growth and the colony characteristics are evaluated: lactose fermenting strains exhibit good growth with red colonies while non-lactose fermenters grow with colourless colonies. Moreover, *E. coli* colonies exhibit a gold metallic sheen.
The 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 *E. faecalis* ATCC 19433 and *S. aureus* ATCC 25923. The growth of non-target strains is partially inhibited.

13-LIMITATIONS OF THE METHODS

- Endo Agar is not a highly selective medium and some yeasts and some Gram-positive bacteria such as enterococci or staphylococci may grow.
- On Endo Agar, swarming of *Proteus* is not inhibited.
- Avoid exposure of the medium to light, as it may lead to photooxidation and decrease the productivity of the medium.
- Overheating of the medium must be avoided, as it may destroy the productivity of the medium.

14 - 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.
- This culture medium is classified as dangerous since contains acid fuchsin, a potential carcinogen. 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 inoculated plates 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.

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 the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).
On exposure to oxygen the plated Endo Agar gradually becomes red due to the oxidation of sulphite and can thus no longer be used.

16 - REFERENCES

1. Endo S. Über ein Verfahren zum Nachweis der Typhus bacillen. Centr f Bakt 1904; 35:109-110.
2. ICMSF. Microorganisms in Foods: their significance and Methods of Enumeration, 2nd ed 1978
3. APHA. Standard Methods for the Examination of Dairy Products, 13th ed 1972
4. APHA. Standard Methods for the Examination of Water and Wastewater, 20th ed 1998

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	2022/08

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

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