

TRYPTONE TRYPTOPHAN MEDIUM

Dehydrated culture medium and ready-to-use tubes

1 - INTENDED USE

Liquid medium to perform the indole test.

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

DEHYDRATED AND READY-TO-USE MEDIUM

Tryptone	10 g
Sodium chloride	5 g
DL-tryptophan	1 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

Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.¹ The indole test is based on the formation of a red colour complex when indole reacts with aldehyde group of p-dimethylaminobenzaldehyde of Kovacs' Reagent, under acidic conditions.

The indole test is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.¹ Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E. coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative).

The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan.² Tryptone Tryptophan medium is prepared according to the formulation included in several ISO Standards.³⁻⁵

Essential growth factors are provided by tryptone which is carbohydrates free, rich in tryptophan and a source of nitrogen, carbon and minerals. The addition of 1 g/L tryptophan enhances the detection of tryptophanase by the indole reaction. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 16 g in 1000 mL of cold purified water. Dissolve the powder by mixing and by heating if necessary. Dispense 5 mL of medium in tubes and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	pale yellow, limpid
Final pH at 20-25 °C	7.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptone Tryptophan Medium	Dehydrated culture medium	4021652	500 g (31.3 L)
Tryptone Tryptophan Medium	Ready-to-use tubes	552165	20 tubes

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, screw capped tubes, sterile loops, Kovacs' Reagent (REF 19171000), ancillary culture media and reagents.

8 - SPECIMENS

The specimens consist of colonies grown on plated media.

9 - TEST PROCEDURE

- Inoculate the Tryptone Tryptophan Medium using a light inoculum of an 18-24 h pure culture and incubate at:
44°C or 37 °C for 24 h for the confirmation test of *E. coli*,
37 °C for 24 h for the confirmation test of *E. coli* O157 according to ISO 16654,³
37 °C for 24 h, for the differentiation of *Salmonella* (generally indole negative) from *E. coli* (indole positive) according to ISO 6579,⁴
30 °C for 48 h, for the biotyping of *Y. enterocolitica* according to ISO 10273,⁵
- Add 1 mL of Kovacs' Reagent and shake gently.
- Examine the upper layer of liquid after about 1 min for the appearance of a red-pink colour.

10 - READING AND INTERPRETATION

Positive result: formation of a pink to red colour within 1 minute (occurring normally within a few seconds)

Negative result: no colour change, the reagent layer remains yellow or slightly cloudy

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 the test strains useful for the quality control.

Positive control: *Escherichia coli* ATCC 25922

Negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection





12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Tryptone Tryptophan Medium are tested with positive and negative strains. Indole positive strains (appearance of a red-pink colour on the upper layer of the medium within few seconds): *E. coli* ATCC 25922, *E. coli* O157 H7 ATCC 43888, *Y. enterocolitica* ATCC 23715, *P. vulgaris* ATCC 13315, *P. rettgeri* ATCC 39944. Indole negative strains (no colour change of the upper layer of the medium within 3 minutes): *S. Typhimurium* ATCC 14028, *S. Enteritidis* ATCC 13076, *E. aerogenes* ATCC 13048, *C. freundii* ATCC 43864.

13 - LIMITATIONS OF THE METHOD

- Indole test is an aid in the differentiation of microorganisms. For complete identification further suitable tests are recommended.
- Change in colour of the Kovacs Reagent from yellow to brown indicates improper storage, which may cause weaker reactions.

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.
- 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Each ready-to-use tube of this culture medium is for single use only.
- 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 Sheets 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

Ready-to-use medium in tubes

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

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 (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 6579, the self-prepared tubes can be stored at +2°C +8°C in the dark for up to 3 months.⁴

16 – REFERENCES

- Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
- MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
- ISO 16654:2001 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli* O157.
- ISO 6579-1:2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.
- ISO 10273:2017 -Microbiology of the food chain. Horizontal method for the detection of pathogenic *Yersinia enterocolitica*.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

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

