

TECHNICAL DATA SHEET

TRYPTOPHAN BROTH

DETECTION OF INDOLE PRODUCTION

1 INTENDED USE

Tryptophan broth allows the culture of microorganisms presenting no particular growth requirements. The media is used to perform the indole production test, used notably in the confirmation of *Escherichia coli*, *Shigella* and *Salmonella*.

The typical composition corresponds to that defined in the standards NF EN ISO 9308-1, NF EN ISO 6579-1, NF EN ISO 16654.

2 PRINCIPLES

Under aerobic conditions, strains possessing a tryptophanase degrade tryptophan to indole. After culturing the strain, the indole produced is revealed by addition of Kovac's reagent.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone 10,0 g
- DL-Tryptophan 1,0 g
- Sodium chloride 5,0 g

pH of the ready-to-use media at 25 °C : 7,5 ± 0,2.

4 PREPARATION

- Dissolve 16,0 g of dehydrated media (BK163) in 1 liter of distilled or demineralized water.
- Slowly stir until complete dissolution.
- Dispense in tubes by adding 3 to 5 mL per tube.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
16,0 g/L

✓ **Sterilization :**
15 min at 121°C

5 INSTRUCTIONS FOR USE

Confirmation of *Escherichia coli* (NF EN ISO 9308-1)

- Transfer a colony taken from the Tergitol 7 agar into a tube of 3 mL of the media prepared as described or use the ready-to-use media (BM076).
- Incubate at 44,0 ± 1,0 °C for 21 ± 3 hours.
- Add 0,2 mL of Kovac's reagent.

✓ **Inoculation :**
A loop of colony

✓ **Incubation :**
21 h at 44 °C

Detection of indole production (*Shigella*, *Salmonella*, *Escherichia coli* O157)

- Take a purified colony from Nutrient Agar and inoculate into a 5 mL tube of Tryptophan broth prepared as described.
- Incubate for 24 ± 3 hours at 37,0 ± 1,0 °C.
- Add 1 mL of Kovac's reagent.

✓ **Inoculation :**
A loop of colony

✓ **Incubation :**
24 h at 37 °C

6 RESULTS

The presence of indole is characterized by the appearance of a red ring in the alcoholic phase of the Kovac's reagent. The ring remains yellow-brown for the negative tests.

See ANNEX 1 : PHOTO SUPPORT.

Escherichia coli presents a positive indole test.

Shigella sonnei and *Salmonella* present a mobile, negative indole test.

The other *Shigella* are variable.

7 QUALITY CONTROL

Dehydrated media : off-white powder, free-flowing and homogeneous.

Prepared media : amber, limpid solution.

Typical culture response after 21 hours of incubation at 44 °C, method qualitative (FD T90-461) :

Microorganisms		Growth	Indole production
<i>Escherichia coli</i>	WDCM 00013	Good, score 2	Positive
<i>Escherichia coli</i>	WDCM 00012	Good, score 2	Positive
<i>Escherichia coli</i>	WDCM 00179	Good, score 2	Positive
<i>Salmonella</i> Typhimurium	WDCM 00031	Good, score 2	Negative
<i>Enterobacter aerogenes</i>	WDCM 00175	Good, score 2	Negative

8 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

Ready-to-use media in tubes : 2-25 °C.

The expiration date is indicated on the label..

Prepared media in tubes (*) : 180 days at 2-25 °C

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media :

500 g bottle BK163HA

Ready-to-use media :

50 x 3mL tubes BM07608

10 BIBLIOGRAPHY

NF EN ISO 9308-1. Septembre 2000. Qualité de l'eau. Recherche et dénombrement des *Escherichia coli* et des bactéries coliformes. Partie 1 : Méthode par filtration sur membrane.

NF EN ISO 16654. Juillet 2001. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157. Modifié par Amendement A1 en Juin 2017.

NF EN ISO 21567. Mars 2005. Microbiologie des aliments. Méthode horizontale pour la recherche de *Shigella* spp..

FD T90-461. Août 2016. Qualité de l'eau - Microbiologie - Contrôle qualité des milieux de culture.

NF EN ISO 6579-1. Avril 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des *Salmonella* - Partie 1 : recherche des *Salmonella* spp..

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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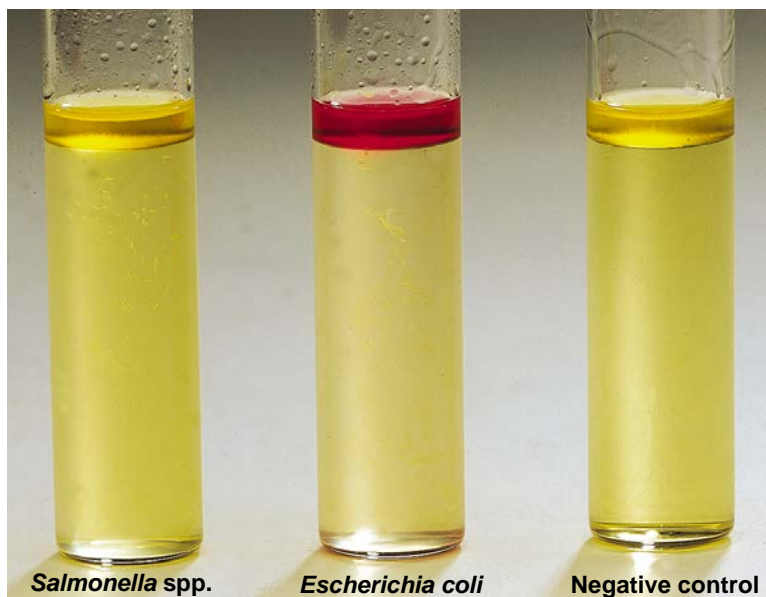
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Tryptophan Broth

Research of indole production.

Results :

Growth obtained after 24 hours of incubation at 37 °C.



Characteristics : the production of indole in *Escherichia coli* is characterized by the appearance of a red ring in the alcoholic phase of the Kovac's reagent (degradation of tryptophan).
The negative control remains colorless to yellowish.