

**TRYPTOPHAN MEDIUM****TM 899**

For detection of indole production

Composition

Ingredients	Gms/Ltr.
Casein enzymatic hydrolysate	10.00
Sodium chloride	5.00
DL-Tryptophan	1.00

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 16gms in 1000ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to room temperature before inoculation.

Appearance: Yellow colour, clear solution without any precipitate

pH (at 25°C): 7.5 ± 0.2

Principle

TRYPTOPHAN MEDIUM is used for detection of indole production. Tryptophan medium water may also be used for differentiation of other bacteria based on indole production. It consists of Casein enzymatic hydrolysate which is a good substrate, which has high levels of the amino acid like **tryptophan**, it also contains some other amino acids. To utilize tryptophan, a bacterium must produce **tryptophanase**. Tryptophanase catalyzes the hydrolysis of tryptophan to ammonia, pyruvate and **indole**. As indole is produced as an end product that is unique to bacteria that produce tryptophanase, one can determine if a given bacteria has tryptophanase activity by testing for the presence of indole. Addition of **Kovac's reagent**, which contains chemicals that react with indole to produce a **red-ring** at the top of the broth. Indole combines with the aldehyde present in the above reagent to give red colour in the alcoholic layer. For complete identification of the organisms, further biochemical confirmation is necessary. Sodium chloride helps maintaining the osmotic balance.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU / ml), on incubation at 35-37°C for 18-24 hours.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Indole reaction
<i>Escherichia coli</i>	25922	10^3	Luxuriant	Positive reaction, red ring at the interface of the medium
<i>Enterobacter aerogenes</i>	13048	10^3	Luxuriant	Negative reaction, no colour development / cloudy ring
<i>Klebsiella pneumoniae</i>	13883	10^3	Luxuriant	Negative reaction, no colour development / cloudy ring

References

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5. 5. MacFaddin J. F., Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore. (2000).
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