Indole Assay Kit (Colorimetric)

LS-K179-100 (100 Tests) • **Store at 4°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

INDOLE is the primary product of tryptophan breakdown by tryptophanase. The indole test is commonly performed on bacteria to classify them on their ability to break down tryptophan to indole. This indole assay kit is based on a modified version of Ehlrich's and Kovac's reagents, which reacts with indole to produce a colored compound at 565 nm. The intensity of this colored compound is directly proportional to the indole in the sample.

Key Features

- Fast and sensitive. Use of 100µL sample. Linear detection range from 3 to 100 µM indole in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the absorbance immediately.

Applications

- Direct Assays: Indole determination in biological samples (e.g. indole produced by indole positive bacteria).

Components

Component	K179-100
	100 Tests
Reagent	12 mL
Standard (10 mM Indole)	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.

Storage

The kit is shipped at RT. Store all components at 4°C upon receiving. Shelf life: 6 months after receipt.

Assay Procedure

Reagent Preparation

Briefly centrifuge Standard tube before opening. Equilibrate all components to room temperature prior assay.

Procedure

1. Standards. Prepare 1 mL of 100 µM Premix by mixing 10 µL of the Standard (10 mM) and 990 µL of the blank medium (e.g. bacterial growth medium). Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + Medium	Indole (µM)
1	200 µL + 0 µL	100
2	100 µL + 100 µL	50
3	50 µL + 150 µL	25
4	0 µL + 200 µL	0

2. Transfer 100 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 100 µL of each sample into separate wells.

3. Add 100 µL Reagent to the four Standards and the Sample Wells. Tap plate to mix briefly and thoroughly. Use of a multi-channel pipettor is recommended.
4. Read optical density at 565 nm (520-590 nm).

Calculations

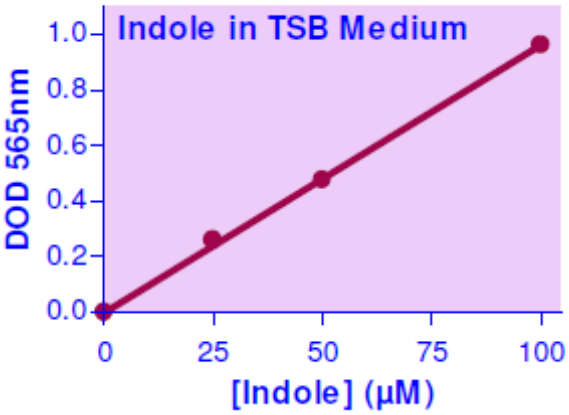
Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the indole concentration of Sample as follows:

$$[\text{Indole}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \quad (\mu M)$$

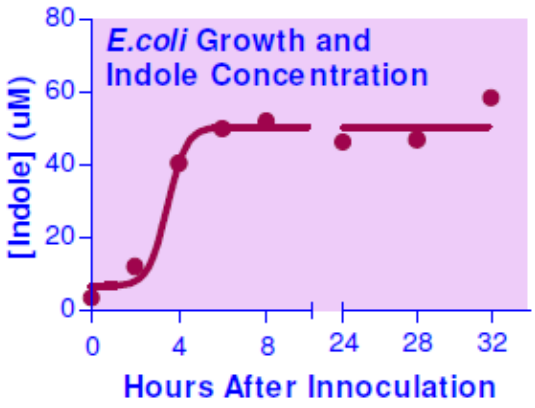
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Media Blank (#4), respectively.

Conversions: 1 µM Indole equals 1.172 mg/dL, or 11.72 ppm.

Sample Data



Indole Standard Curve in TSB Medium
Standard curve of indole concentrations in TSB medium



E.coli Growth and Indole Concentration
E.coli cells inoculated into 5 mM Tryptophan medium.
Medium samples taken every two hours.

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