Technical Manual

ChromaDazzle Indole Assay Kit

Catalogue Code: BA0046

Pack Size: 100 assays

Research Use Only

DESCRIPTION

INDOLE is the primary product of tryptophan breakdown by tryptophanase. The indole test is a commonly performed on bacteria to classify them on their ability to break down tryptophan to indole.

The Assay Genie ChromaDazzle 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 100 µL 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 in biological samples (e.g. indole produced by indole positive bacteria).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent: 12 mL
Standard: 100 µL (10 mM Indole)

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

Precautions: reagents are for research use only. Briefly centrifuge Standard tube before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Procedure using 96-well plate

1. *Standards*. Prepare 1 mL of 100 µM Premix by mixing 10 µL of the 10 mM Standard 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.
4. Read optical density at 565 nm (520-590 nm).

CALCULATION

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:

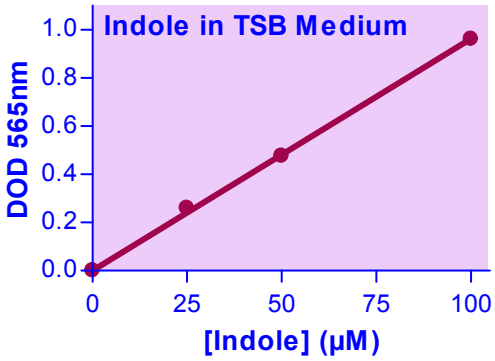
$$[\text{Indole}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \quad (\mu M)$$

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

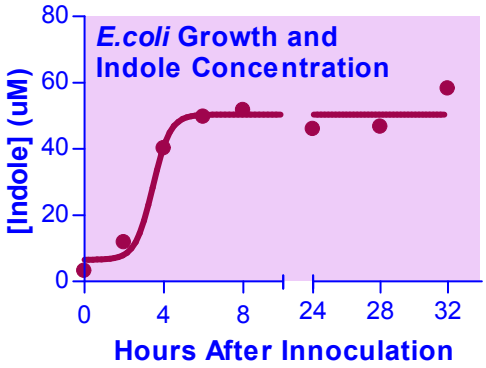
Conversions: 1 µM Indole equals 1.17 mg/dL, or 11.7 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



Indole Standard Curve in TSB Medium



***E.coli* Growth and Indole Concentration**

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

LITERATURE

1. Kuczyńska-Wiśnik, D., et al (2010). Escherichia coli heat-shock proteins IbpA and IbpB affect biofilm formation by influencing the level of extracellular indole. Microbiology 156: 148-157.
2. Xu, Z.R., et al (2006). Effects of fructooligosaccharide on conversion of L-tryptophan to skatole and indole by mixed populations of pig fecal bacteria. J Gen Appl Microbiol 48: 83-89.
3. Bansal, T., et al (2009). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proc Natl Acad Sci 107: 228-233.

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