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Product Information

Indole Assay Kit

Catalog Number **MAK326**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description
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.

The 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. Linear detection ranges from 3 to 100 μM indole in a 96-well plate assay.

This kit is suitable for indole determination in biological samples (e.g. indole produced by indole positive bacteria).

Components
The kit is sufficient for 100 colorimetric assays in 96-well plates.

Reagent 12 mL
Catalog Number MAK326A

Standard (10 mM Indole) 100 μL
Catalog Number MAK326B

Reagents and Equipment Required but Not Provided.
- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped at room temperature. Store all components at 2-8 °C upon receiving.

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

Procedure
Indole 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 Table 1.

Table 1.
Preparation of Indole Standards

Tube	100 µM Premix	Growth 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

Assay Reaction
1. Transfer 100 μL standards into separate wells of a clear, flat-bottom 96-well plate.
2. 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.
3. Measure the absorbance at 565 nm (A_{565}).

Results
Subtract the blank value (standard tube #4) from the standard values and plot the A_{565} against standard concentrations. Determine the slope and calculate the indole concentration of Sample as follows:

$$\text{Indole} = \frac{(A_{565})_{sample} - (A_{565})_{blank}}{\text{Slope } (\mu M^{-1})} \quad (\mu M)$$

$(A_{565})_{sample}$ = the optical density of the sample
$(A_{565})_{blank}$ = the optical density of the media blank (standard tube #4)

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

Figure 1.
Standard curve of indole concentrations in TSB medium

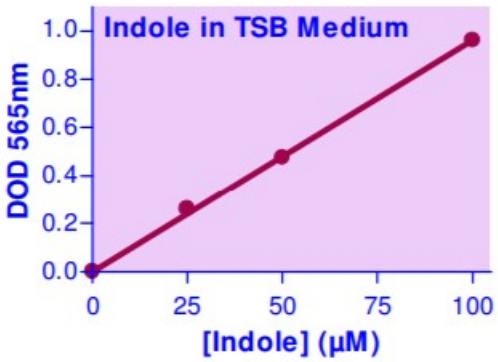
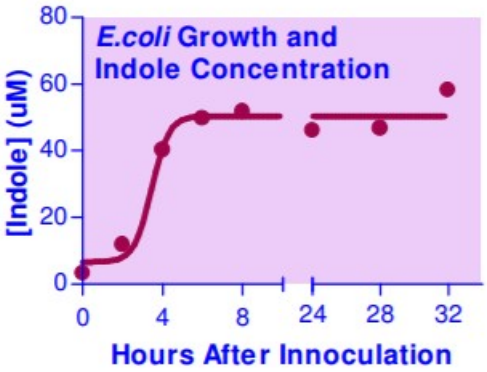


Figure 2.
E.coli cells inoculated into 5 mM Tryptophan medium. Medium samples taken every two hours.



References

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3. Bansal, T., et al., The bacterial signal indole increases epithelial cell tight-junction resistance and attenuates indicators of inflammation. *Proc. Natl. Acad. Sci. USA.,* **107**, 228-233 (2009).

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