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ab272536
Indole Assay Kit

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Indole Assay Kit datasheet:

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For quantitative determination of Indole concentration in biological samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Indole is the primary product of tryptophan breakdown by tryptophanase. 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.

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.

2. Protocol Summary

Prepare all reagents and samples as instructed

Add standards and samples to appropriate tubes.

Add reagent to samples and standards.

Read absorbance at 565 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent	12 mL	+4°C
Standard (10 mM)	100 µL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H2O
- Multi-channel pipette
- 1.5 mL tubes
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 520-590 nm (peak absorbance is at 565 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Briefly centrifuge Standard tube before opening.
- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 100 assays.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

Premix Standard:

10.1.1 Prepare 1 mL of 100 µM Premix by mixing 10 µL of 10 mM Indole Standard and 990 µL of blank medium (e.g. bacterial growth medium).

10.1.2 Dilute standards in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix (µL)	Medium (µL)	Indole Conc. (µM)
1	200	0	100
2	100	100	50
3	50	150	25
4	0	200	0

11. Sample Preparation

Δ **Note:** The Assay will detect indole in urine samples; however, the reagent also reacts with urobilinogen in the urine. It does not distinguish between the two.

Δ **Note:** The acidity of the reagent often causes serum samples to precipitate which greatly interferes with the assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1.1 Add 100 µL of standards and each sample to separate wells.
12.1.2 Add 100 µL of reagent to each sample and standard well. Tap plate to mix briefly and thoroughly.
12.1.3 Read OD at 520-590 nm (peak 565 nm).

Δ Note: Use of a multi-channel pipettor is recommended.

Δ Note: The OD of the reaction mixture slowly decreases over time. We would recommend reading the OD within 10 minutes of adding the reagent.

13. Calculations

13.1.1 Subtract Standard #4 (Medium) OD value from the remaining Standards (#1, 2, 3) OD values and plot ΔOD against standard concentrations.

13.1.2 Determine the slope.

13.1.3 Calculate the indole concentrations of samples as follow:

$$[\text{Indole}] \text{ in } \mu M = \frac{OD_{Sample} - OD_{Blank}}{Slope}$$

- OD_{SAMPLE} is optical density reading of the Sample.
- OD_{BLANK} is optical density reading of the Medium Blank (#4).
- Slope is the slope of the standard curve (μM^{-1})

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

Figure 1. Example of Indole Assay Kit standard curve in TBS medium.

Figure 2. Example of *E. coli* growth and Indole concentration. *E.coli* cells inoculated into 5 mM Tryptophan medium. Medium samples taken every two hours.

15. Notes

Technical Support

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