Product Manual

Indole Assay Kit

Catalog Number

MET-5122 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Indole is an aromatic heterocyclic organic compound made up of a six-membered benzene ring fused to a five-membered pyrrole ring. Indole is widely distributed in nature and can be synthesized by a number of bacteria. Indole is biosynthesized by a seven-step metabolic pathway in bacteria, fungi, archaea, and some plants known as the shikimate pathway. Acting as an intercellular signal, indole controls various bacterial physiological processes, including plasmid stability, resistance to drugs, spore formation, biofilm formation, and virulence. Indole is an intermediate in production of tryptophan in these organisms, and when indole production is required, it can be made from tryptophan breakdown using tryptophanase. In humans, bacteria in the gut, also known as the human gastrointestinal microbiota, tryptophan catabolism leads to the synthesis of indole and other important bioactive derivatives. In intestinal L cells, indole produced from gut bacteria causes the secretion of glucagon-like peptide-1 which acts as a ligand for aryl hydrocarbon receptor. Indole is also converted to indoxyl sulfate in the liver which is linked to increased oxidative stress, increased muscle cell proliferation, increased aortic wall thickness, increased aortic wall calcification, and chronic kidney disease.

Cell Biolabs' Indole Assay Kit is a simple colorimetric assay that measures the total amount of free indole present in urine, fecal, and bacterial samples in a 96-well microtiter plate format. Unlike the traditional method of indole detection using Kovac's reagent which is less specific since it also detects 3-methylindole (skatole) found in bacterial samples, Cell Biolabs' Indole assay kit uses a proprietary chemistry to form a colored product from indole (but not skatole or other biologically relevant derivatives). This colored product is then measured with a standard 96-well spectrophotometric plate reader, and samples are compared to a known concentration of indole standard within the 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, indole standards, and unknown samples. Sample indole concentrations are determined by comparison with a known indole standard. The kit has a detection sensitivity limit of 31 µM indole.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit
3. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
4. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
5. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit

Kit Components (shipped on blue ice)

1. Indole Standard (Part No. 51221B): One 1 mL vial at 10 mM.
2. Assay Reagent A (Part No. 51222A): One 3 mL bottle
3. Assay Reagent B (Part No. 51223A): One 5 mL bottle
4. Assay Reagent C (Part No. 51224A): One 13 mL bottle.

Materials Not Supplied

1. 70% ethanol
2. Distilled or deionized water
3. Standard 96-well clear microtiter plate

Storage
Upon receipt, store the kit at 4°C.

Preparation of Samples
- Fecal Samples: Sonicate or homogenize fecal samples in 70% ethanol and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions as necessary into 70% ethanol.
- Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary into 70% ethanol.
- Bacterial Cultures: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary into 70% ethanol.

Note: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

Preparation of Standard Curve
Prepare fresh Indole Standards before use by diluting in deionized water according to Table 2.

Standard Tubes	10 mM Indole Solution (µL)	70% Ethanol (µL)	Indole (µM)
1	100	400	2000
2	250 of Tube #1	250	1000
3	250 of Tube #2	250	500
4	250 of Tube #3	250	250
5	250 of Tube #4	250	125
6	250 of Tube #5	250	62.5
7	250 of Tube #6	250	31.2
8	0	250	0

Table 2. Preparation of Indole Standards.

Assay Protocol
Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each sample replicate requires two paired wells, one to be treated with Assay Reagents A, B, and C (Reaction) and one with water in place of Assay Reagents (Control) to serve as a background control.

1. Add 100 µL of each Indole Standard or unknown sample into wells of a 96-well microtiter plate.
2. Add 25 µL of Assay Reagent A to the standards and one half of the paired sample wells. Mix the well contents thoroughly.
3. Add 50 µL of Assay Reagent B to the standards and one half of the paired sample wells. Mix the well contents thoroughly.
4. Incubate the reaction plate for 15 minutes at room temperature.
5. Add 125 µL of Assay Reagent C to the standards and one half of the paired sample wells. Mix the well contents thoroughly.
6. Add 200 µL of deionized water to the other half of the paired sample wells and mix the well contents thoroughly.

7. Incubate the reaction plate for 15 minutes at room temperature.
8. Read the plate at 540 nm using a microplate spectrophotometer.

Example of Results

The following figures demonstrate typical Indole Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

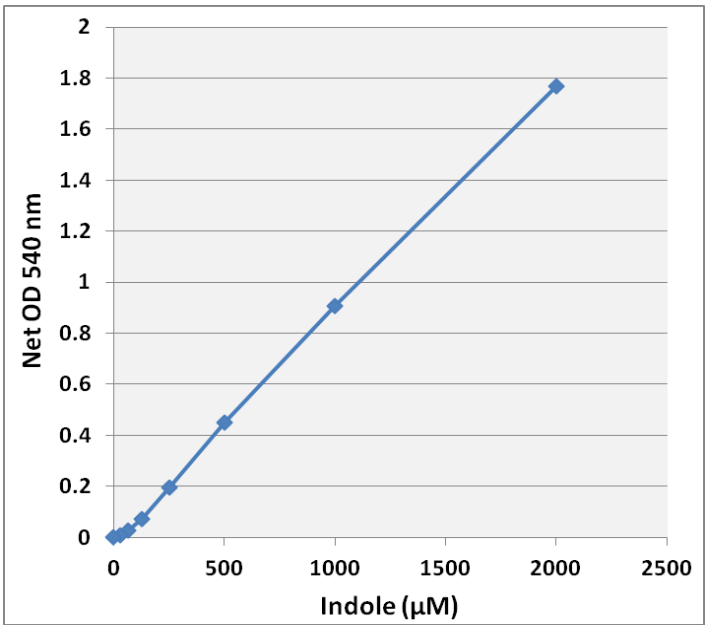

Figure 2: Indole Standard Curve.

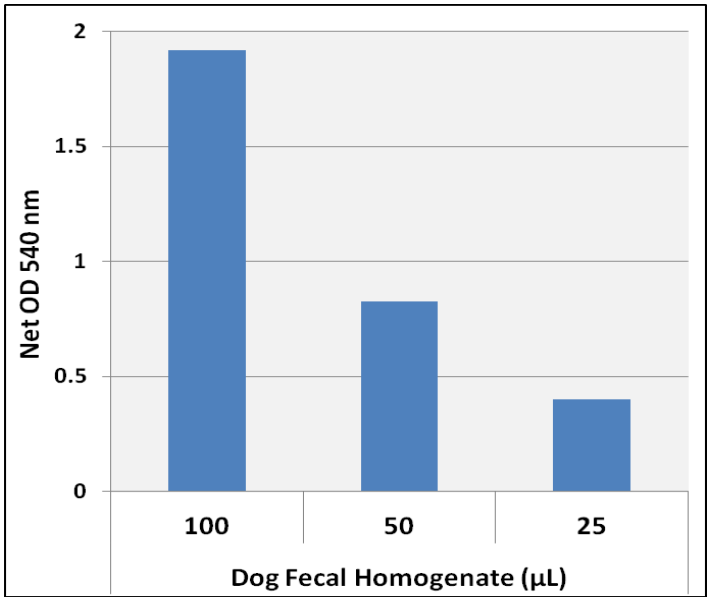

Figure 3: Indole Detection in Dog Feces. Dog fecal homogenate was prepared according to the Preparation of Samples section and then tested according to the Assay Protocol.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Assay Reagents "A_{Ctrl}" from the sample well values containing Assay Reagents "A_{Rxn}" to obtain the difference. The absorbance difference is due to the production of colored product:

$$\Delta A = A_{Rxn} - A_{Ctrl}$$

6. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of indole present in the sample. Only use values within the range of the standard curve.

References

1. Lee, J-H, Lee, J (2010). *FEMS Microbiol Rev*. **34**: 426–44.
2. Zhang LS, Davies SS (2016). *Genome Med*. 8: 46-64.
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4. Martineau B and LaFlamme DP (2002). *Res. Vet. Sci*. **72**:223-227.
5. Mine M, Masaki N, Nagatoma Y, Takase B, and Adachi T. (2018) *Eur. Heart J*. **39**:488(P2512)
6. Turner JM. (1961) *Biochem. J.* **78**:790-792.

Warranty

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