ABS_Bio™ Indican Colorimetric Detection Kit

(Cat# K334-100; 100 assays; store kit at -20℃)

Introduction

The essential amino acid tryptophan is converted to indole by intestinal bacteria. Most indoles are excreted in the feces. The remainder is absorbed, metabolized by the liver, and excreted as indican in the urine. High amounts of indican can indicate overgrowth of bacterial organisms in the intestines. Elevated levels are considered as an indicator of intestinal toxemia and overgrowth of anaerobic bacteria. Conditions that lead to excess urine Indican include: Maldigestion and/or malabsorption of protein; Bacterial overgrowth in the small and/or large intestine; Liver Dysfunction.

The ABS_Bio™ Indican Colorimetric Detection Kit provides a simple and direct procedure for measuring indicant levels in urine. In this assay, indican is treated with improved Ehrlich's reagent at acidic condition to form a colored complex. The intensity of the color measured at 480 nm is directly proportional to the amount of indican in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection range of 1.2-1000 µM (0.03-25 mg/dL). It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (100 tests)

Reagent A : 12 mL Reagent B: 0.6 mL Indican Standard (10 mM): 0.8 mL

Storage and Handling: Store Reagent A at room temperature, others at -20℃. Shelf Life: 12 months after receipt.

Protocol

1. Sample preparation

Urine and other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample to ensure the readings are within the standard curve range. Serum or plasma sample need add same volume of 10% TCA to deproteinizing, mix and incubate 10 min, spin down at 14K rpm for 5 min, correct supernatant to assay. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.
Transfer 50 µL sample into the 96-well clear flat bottom plate in duplicate (one as test sample, another as sample blank).

2. Standard Preparation

Transfer 40 µL 10 mM indican standard into 360 µL dH_2O to generate a 1 mM indican standard, then following the table to generate 1, 0.6, 0.3, 0.1 and 0 mM indican standards.

1mM Indican std(µL)	dH_2O(µL)	final indican concentration mM (mg/dL)
200	0	1 (25.12)
120	80	0.6 (15.08)
60	140	0.3 (7.54)
20	180	0.1 (2.51)
0	200	0 (0.0)

Transfer 50 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 100 µL Reagent A and 5 µL Reagent B for each reaction (samples & standards).

Transfer 100 µL prepared working reagent into sample and standard reaction well. Directly add 100 µL Reagent A into each sample blank wells. Tap plate to mix well. Incubate 15 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 480 nm (460-500 nm).

5. Calculation

Average the duplicate OD480 nm reading for standard. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the indican concentration of the standards. Determine the slope from the standard curve and calculate the indican concentration of samples.

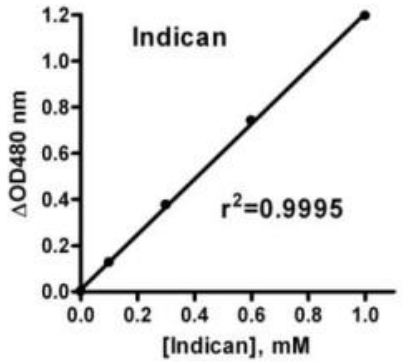
[Indican]= (OD_{sample}-OD_{blank})/Slope x *n* (mM)

OD_{sample} and OD_{blank} are related optical density of the sample and dH_2O. *n* is the sample dilution factor.

Note: if has sample blank, the sample blank reading must be subtracted from sample readings.

Conversions: 1 µM indican equals 25.1 µg/dL (0.0251 mg/dL).

Typical Standard Curve



Indican standard in 96 wells-plate assay.
Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.0012 mM, and linear detection range up to 1 mM in 96-well plate colorimetric assay. Sensitivity was determined as 0.1 μM. Samples with values above 1 mM (25 mg/dL) should be dilute with dH_2O, re-assayed, and multiply results by dilution factor.

Interferences

Colored sample should run sample blank control parallel; sample with high concentration protein should be deproteinizing prior the assay.

References

Curzon, G. et al. 1962, Clin Chim Acta 7:657-663
Novis, BH. Et al. 1971, S Afr Med J. 45(41):1167-70.
Todd, J. et al. 1979, Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Phil, Pa. pp 592-3

Related Products:

Glycerol Detection Kit (#K315-100)
Triglyceride Detection Kit (#K316-100)
NAD/NADH Detection Kit (#K120-100)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
Uric Acid Detection Kit (#K152-200)
Copper Colorimetric Detection Kit (#K330-200)
Magnesium Colorimetric Detection Kit (#K332-200)
Zinc Colorimetric Detection Kit (#K333-200)
Urea Detection Kit (#K158-200)
Heme Detection Kit (#K169-200)
Lactate Detection Kit (#K207-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphatase Detection Kit (#K196-500)
ADP Detection Kit (#K134-100)
Choline Detection Kit (#K310-100)
TBARS Detection Kit (#K145-100)
Pyruvate Detection Kit(#K150-100)
Hemoglobin Detection Kit (#K168-200)
Sulfate Detection Kit (#K149-200)
Iron Colorimetric Detection Kit (#K329-200)
Calcium Colorimetric Detection Kit (#K301-200)
Urinary Indican Test Kit (#T158-20)