QuantiChrom™ Indican Assay Kit (DIDC-100)

Quantitative Colorimetric Determination of Urinary Indican

DESCRIPTION

Indican (indoxyl sulfate) is a putrefaction product resulting from bacterial deconjugation of dietary tryptophan to indole in the small intestine. A large body of evidence supports the use of urinary indican testing as a beneficial point-of-care screening tool for dysbiosis, small intestinal bacterial overgrowth (SIBO), constipation, malabsorption, intestinal mucosal permeability etc. Common microorganisms that contribute to positive indican tests include *Salmonella*, *Shigella*, *Campylobacter jejuni*, *yersinia enterocolitica*, *Citrobacter freundi*, *Citrobacter diversus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, certain strains of *Escherichia coli*, *Staphyloccocus aureus*, some strains of bacteriodes, *Clostridium difficile*, *Candida albicans*, other candida species etc.

Traditional Indican test using Obermayer's reagent gives only qualitative results. BioAssay Systems' quantitative indican assay kit is based on an improved Curzon and Walsh method. In this assay, indican reacts with a chromogen. The color intensity of the product at 480nm is directly proportional to the indican concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 0.2 - 20 mg/dL (8-800 µmoles/L).

Fast and high-throughput. Homogeneous "mix-incubate-measure" type assay. Assay takes only 10 min. Can be readily automated for processing thousands of samples per day.

APPLICATIONS

Quantitative direct determination of indican in urine samples.

KIT CONTENTS

Catalog#	Reagent A	Reagent B	Standard	Tests (96-well)	Tests (Cuvette)
DIDC-100	20 mL	1.5 mL	1.5 mL 30 mg/dL	100	20
DIDC-01K	200 mL	15 mL	15 mL 30 mg/dL	1000	200
For bulk orders of >1000 mL, please inquire for a quote.					

Storage conditions: This product is shipped at room temperature. Store Reagent A at room temperature and other components at -20°C. Shelf life of 12 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Please note:

(1). Reagent A contains hydrochloric acid (HCl). Wear appropriate gloves, protective clothing and eyewear and follow safe laboratory practices.

(2). Patient samples. No alcohol the night before; a high protein meal the night before; no iodine or bile supplements taken in high doses 3 - 4 days prior to testing; second urination of the day. If not assayed immediately, samples can be frozen for up to 10 days.

ASSAY PROCEDURE FOR 96-WELL PLATE READER

Use clear flat-bottom 96-well plates. Prior to assay, bring all reagents to room temperature. Vortex Reagent B briefly. Use a multichannel pipettor when assaying a large number of samples in one run.

1. Transfer 50 µL urine samples into separate wells of the 96-well plate.
2. Add 140 µL Reagent A to each assay well. Tap plate to mix. Measure OD_{480nm} (OD_O) on a plate reader.
3. Add 10 µL Reagent B to each assay well. Tap plate to mix. Incubate 5 min and read OD_{480nm} (OD_S).
4. Add 10 µL provided standard to each assay well. Tap plate to mix. Incubate 5 min and read OD_{480nm} (OD_{STD}).

CUVETTE ASSAY PROCEDURE

The above procedure can be scaled up or down. The following procedure is used for a standard 1 mL cuvette.

1. Transfer 250 µL urine samples into separate cuvettes.
2. Add 700 µL Reagent A to all samples. Tap cuvette to mix. Measure OD_{480nm} (OD_O) on a spectrophotometer.
3. Add 50 µL Reagent B to all cuvettes. Tap cuvette to mix. Incubate 5 min and read OD_{480nm} (OD_S).
4. Add 50 µL provided standard. Tap cuvette to mix. Incubate 5 min and read OD_{480nm} (OD_{STD}).

CALCULATION

96-well plate assay:

$$[\text{Indican}] = \frac{OD_S - OD_o}{OD_{STD} - OD_S} \times 5 \times n \quad (mg/dL)$$

Cuvette assay (fixed light path-length, volume corrections made):

$$[\text{Indican}] = \frac{OD_S - 950/1000 \times OD_o}{1050/1000 \times OD_{STD} - OD_S} \times 5 \times n$$

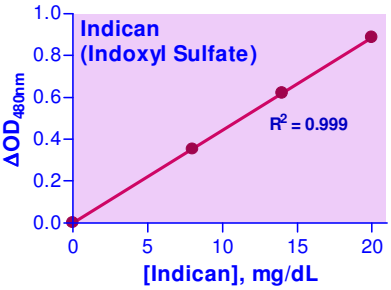
$$= \frac{OD_S - 0.95 \times OD_o}{1.05 \times OD_{STD} - OD_S} \times 5 \times n \quad (mg/dL)$$

where OD_o, OD_S and OD_{STD} are OD_{480nm} values of the Sample blank, endogenous indican in the urine sample and total indican (endogenous + 5 mg/dL spiked indican), respectively. If calculated indican concentration is higher than 20 mg/dL, dilute Sample in water and repeat assay. Multiply the results by the dilution factor *n*.

Conversion factor: 1 mg/dL indican (indoxyl sulfate potassium salt) = 39.8 µmoles/L, or 10 ppm.

MATERIAL REQUIRED BUT NOT PROVIDED

For 96-well plate assays: pipetting devices, clear flat bottom 96-well plates and plate reader. For cuvette assays: pipetting devices, cuvettes and spectrophotometer (480nm).



Linearity of indican assay in 96-well plate assay

LITERATURE

1. Niwa T. (2010). Uremic toxicity of indoxyl sulfate. Nagoya J Med Sci. 72(1-2):1-11. *Review article*.
2. Novis BH. et al (1971). The value of estimating urinary indican. S Afr Med J. 45(41):1167-70.
3. Mayer PJ and Beeken WL (1975). The role of urinary indican as a predictor of bacterial colonization in the human jejunum. Am J Dig Dis. 20(11):1003-9.