

Indican Assay Kit (Colorimetric)

LS-K320-100, -1000 (100, 1000 Tests) • See Storage Conditions Below



Introduction

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 freundii, Citrobacter diversus, Klebsiella pneumoniae, Pseudomonas aeruginosa, certain strains of Escherichia coli, Staphylococcus aureus, some strains of bacteriodes, Clostridium difficile, Candida albicans, other candida species, etc. Traditional Indican test using Obermayer's reagent gives only qualitative results. This 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 μ moles/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.

Components

Component	K320-100	K320-1000
	100 Tests (96-well) 20 Tests (Cuvette)	1000 Tests (96-well) 200 Tests (Cuvette)
Reagent A	20 mL	200 mL
Reagent B	1.5 mL	15 mL
Standard (30 mg/dL)	1.5 mL	15 mL

Materials Not Supplied

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).

Storage

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.

FOR RESEARCH USE ONLY! Not for use in humans.

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Precautions

- (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

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 μ L urine samples into separate wells of the 96-well plate.
2. Add 140 μ L Reagent A to each assay well. Tap plate to mix. Measure OD_{480nm} (OD_o) on a plate reader.
3. Add 10 μ L Reagent B to each assay well. Tap plate to mix. Incubate 5 min and read OD_{480nm} (OD_s).
4. Add 10 μ L 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 μ L urine samples into separate cuvettes.
2. Add 700 μ L Reagent A to all samples. Tap cuvette to mix. Measure OD_{480nm} (OD_o) on a spectrophotometer.
3. Add 50 μ L Reagent B to all cuvettes. Tap cuvette to mix. Incubate 5 min and read OD_{480nm} (OD_s).
4. Add 50 μ L provided standard. Tap cuvette to mix. Incubate 5 min and read OD_{480nm} (OD_{STD}).

Calculations

96-Well Plate Assay:

$$[\text{Indican}] = \frac{\text{OD}_s - \text{OD}_o}{\text{OD}_{\text{STD}} - \text{OD}_s} \times 5 \times n \quad (\text{mg/dL})$$

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

$$\begin{aligned} [\text{Indican}] &= \frac{\text{OD}_s - 950/1000 \times \text{OD}_o}{1050/1000 \times \text{OD}_{\text{STD}} - \text{OD}_s} \times 5 \times n \\ &= \frac{\text{OD}_s - 0.95 \times \text{OD}_o}{1.05 \times \text{OD}_{\text{STD}} - \text{OD}_s} \times 5 \times n \quad (\text{mg/dL}) \end{aligned}$$

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 μ moles/L, or 10 ppm.

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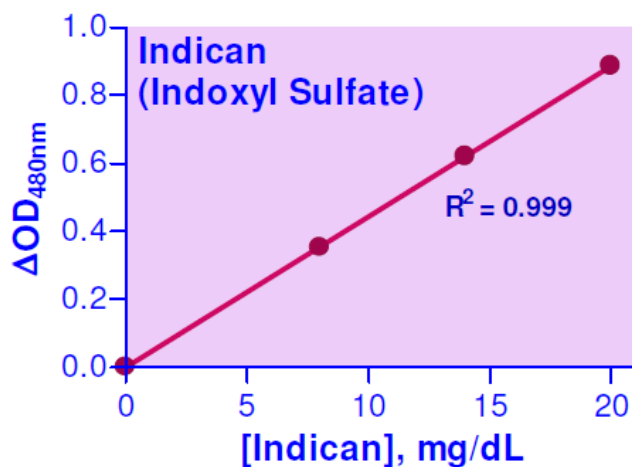
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Sample Data



Linearity of indican assay in 96-well plate assay

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