

Indican Assay Kit (Colorimetric)

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(Catalog # K2083-100; 100 assays; Store at -20 °C)

I. Introduction:

Indican, also known as indoxyl sulfate is a putrefaction product resulting from the bacterial deconjugation of dietary tryptophan in the small intestine. Most of the indican is excreted through feces, while the remainder is absorbed, metabolized and excreted in urine. Indican levels are directly associated with the bacterial activity in the intestine and its levels are low in normal urine. However, an increase in urine indican levels can indicate inefficient protein digestion such as bowel obstructions, stomach disorder, intestinal disorder, pancreatic insufficiency or overgrowth of the gut anaerobic bacteria. Thus, urinary indican levels can be a beneficial screening tool for dysbiosis, small intestine anaerobic bacterial overgrowth, malabsorption, maldigestion, intestinal mucosal permeability etc. **BioVision's Indican Assay Kit** is a quantitative assay for measuring indican concentration in urine samples. In this assay, indican reacts with p-dimethylaminobenzaldehyde (DMAB) in the presence of an acid to yield an orange-colored compound measured at 480 nm. The intensity of the color is directly proportional to the indican concentration in the sample(s). The assay is simple, easy to perform and is reliable. The assay detection range is between 0.5-30 mg/dl.



II. Application:

- To measure the indican concentration in urine samples.

III. Sample Type:

- Urine

IV. Kit Contents:

Components	K2083-100	Cap Code	Part Number
Indican Assay Buffer	25 ml	WM	K2083-100-1
DMAB (in DMSO)	5 ml	Amber	K2083-100-2
Indican Standard (30 mg/ml)	100 µl x 2	Yellow	K2083-100-3

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- dH₂O

VI. Storage Conditions and Reagent Preparation:

Store the unopened kit at -20 °C. Briefly centrifuges all small vials prior to opening. Read the entire protocol before performing the assay.

- Indican Assay Buffer:** Store at 4 °C. Bring to room temperature (RT) before use. **Note:** Indican Assay Buffer contains hydrochloric acid. **Wear gloves and follow safe laboratory practices when handling.**
- DMAB:** Divide into aliquots in amber vials and store at -20 °C, protected from light. Bring to RT before use.
- Indican Standard (30 mg/ml):** Store at -20 °C, protected from light. Bring to RT before use. Once opened, use within 2 months. For the assay, prepare a 100-fold dilution of the 30 mg/ml Indican Standard stock to prepare 30 mg/dl Indican Standard solution by adding 2 µl of 30 mg/ml Indican standard stock to 198 µl dH₂O and mix well. **Note:** Prepare 30 mg/dl diluted Indican Standard immediately before the assay and discard the unused, diluted Indican Standard.

VII. Indican Assay Protocol:

1. Urine Sample Preparation: Use a sterile container to collect the urine sample. Remove any insoluble particles by centrifugation at 1,000 x g and 4°C for 15 min. Collect the supernatant for the assay. **Note:** Collect the first morning urine sample in clean container.

For each sample type, prepare two parallel wells labeled as **Unspiked Sample** and **Spiked Sample** respectively. In **Unspiked Sample** well, add 50 µl of urine sample into the well(s) of a 96-well clear plate. In **Spiked Sample** well, add 50 µl of urine sample and 10 µl of diluted Indican Standard (30 mg/dl) into the well(s) of a 96-well clear plate. Prepare a **Background Control** well containing 60 µl dH₂O. Adjust the volumes of Unspiked Sample and Background Control wells to 60 µl/well with dH₂O and mix well. **Note:** Spiked Sample well has an Indican Standard concentration of 5 mg/dl (i.e. 10 µl of 30 mg/dl diluted Indican Standard in 60 µl total volume).

The schematic of the wells are shown below

	[Unspiked Sample]	[Spiked Sample]	[Background Control]
Urine	50 µl	50 µl	-
Diluted Indican Standard (30 mg/dl)	-	10 µl	-
dH ₂ O	10 µl	-	60 µl

2. Reaction Mix Preparation: Add 140 µl Indican Assay Buffer to all wells including Unspiked Sample, Spiked Sample, and Background Control and mix well. Add 20 µl DMAB to all wells. **Mix well and incubate at 37 °C for 60 min, protected from light.** The total reaction volume in each well is 220 µl/well.

3. Measurement: Measure the plate at OD 480 nm at 37 °C in end-point mode.

4. Calculation: For both Unspiked and Spiked Sample wells, calculate the corrected absorbance signal ($OD_{corrected}$) by subtracting the Background Control OD (OD_{BC}) reading from each of the corresponding Sample OD readings i.e ($OD_{Unspiked\ corrected} = OD_{Unspiked} - OD_{BC}$) and ($OD_{Spiked\ corrected} = OD_{Spiked} - OD_{BC}$).

Determine the amount of Indican (mg/dl) in the Unspiked Sample wells using the following formula:

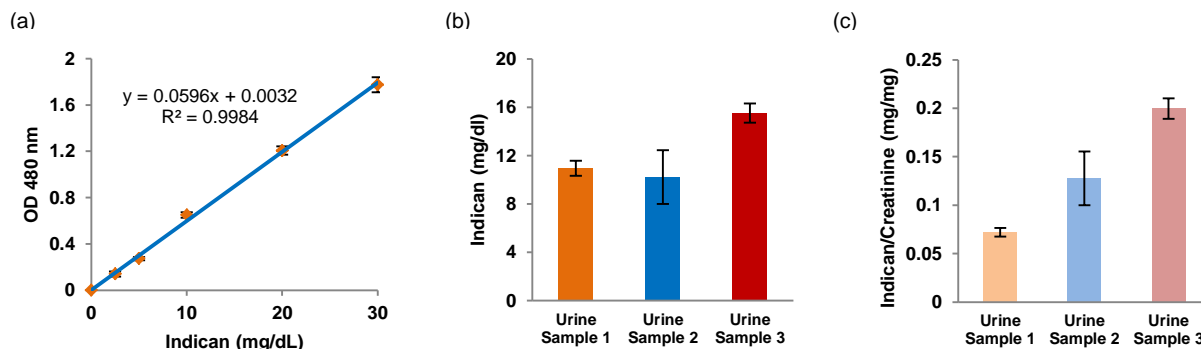
$$\text{Indican amount in sample} = \left(\frac{OD_{(unspiked\ corrected)}}{(OD_{(spiked\ corrected)} - OD_{(unspiked\ corrected)})} \right) * 5 * 1.2 \left(\frac{mg}{dL} \right)$$

Where: 5 mg/dl is Indican Standard Spiked in samples

1.2 is the dilution factor (i.e 50 μ l urine in 60 μ l volume)

Notes:

- 1) The concentration of Indican in Sample(s) can be reported as mg of Indican per mg creatinine to normalize the results combined with creatinine assay.
- 2) If calculated indican concentration is higher than 20 mg/dl, dilute sample in dH₂O and repeat the assay. Record the dilution factor and use it for the final calculation.



Figures: (a) Indican Standard Curve. (b) Indican amounts (mg/dl) in human urine samples (50 μ l). Calculation is based on samples spiked with 5 mg/dl Indican Standard. (c) Results were normalized as mg of Indican per mg creatinine by using Creatinine Colorimetric/Fluorometric Assay Kit (BioVision Cat# K625-100). Assays were performed following the kit protocol.

VIII. Related Products:

- Creatinine Colorimetric/Fluorometric Assay Kit (K625)
- Creatine Colorimetric/Fluorometric Assay Kit (K635)
- Sarcosine Colorimetric/Fluorometric Assay Kit (K636)
- Urea Colorimetric Assay Kit (K375)

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