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Product Information

Indican Assay Kit

Catalog Number **MAK128**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Indican (indoxyl sulfate) is a putrefaction product resulting from bacterial deconjugation of tryptophan in the small intestine. Indican is water soluble and excreted through urine. Increases in urine indican can indicate protein maldigestion or malabsortion, or the overgrowth of gut anaerobic bacteria. Indican levels in urine can also increase in response to a high protein diet.

The Indican Assay kit provides a simple and direct procedure for measuring indican levels in urine. This assay is based on an improved Curzon and Walsh method in which the reaction of indican with *p*-dimethylaminobenzaldehyde results in a colorimetric product measured at 480 nm. This assay has a linear detection range between 0.2–20 mg/dL in the 96 well plate assay.

Components

The kit is sufficient for 100 assays in 96 well plates.

Reagent A Catalog Number MAK128A	20 mL
Reagent B Catalog Number MAK128B	1.5 mL
Standard, 30 mg/dL Catalog Number MAK128C	1.5 mL

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature. Storage at –20 °C, protected from light, is recommended. If desired, Reagent A can be stored at room temperature.

Procedure

Prior to assay, bring all reagents to room temperature. Vortex Reagent B briefly. When assaying large numbers of samples, it is recommended to use a multi-channel pipettor.

Assay Reaction in 96 well plate

1. Transfer 50 µL of urine samples into separate wells of a 96 well plate.

2. Add 140 µL of Reagent A to each assay well and tap plate lightly to mix. Measure the absorbance at 480 nm (A_{480}). This is the blank reading [$(A_{480})_{blank}$].

3. Add 10 µL of Reagent B to each assay well and tap plate lightly to mix. Incubate 5 minutes and measure (A_{480}). This is the sample reading [$(A_{480})_{sample}$].

4. Add 10 µL of provided Standard to each well. Tap plate lightly to mix. Measure the absorbance (A_{480}). This is the standard reading [$(A_{480})_{standard}$].

Assay Reaction in Cuvettes

1. Transfer 250 µL of urine samples into separate cuvettes.

2. Add 700 µL of Reagent A to each cuvette. Measure the absorbance at 480 nm (A_{480}). This is the blank reading [$(A_{480})_{blank}$].

3. Add 50 µL of Reagent B to each cuvette and tap cuvette lightly to mix. Incubate 5 minutes and measure (A_{480}). This is the sample reading for endogenous indican [$(A_{480})_{sample}$].

4. Add 50 µL of provided Standard to each cuvette. Tap cuvette lightly to mix. Measure the absorbance (A_{480}). This is the standard reading [$(A_{480})_{standard}$].

Results

Calculations

Concentration of Indican for 96 well plate assay:

$$(\text{mg/dL}) = \frac{(A_{480})_{sample} - (A_{480})_{blank}}{(A_{480})_{standard} - (A_{480})_{sample}} \times 5 \times n$$

Concentration of Indican for cuvette assay:

$$(\text{mg/dL}) = \frac{(A_{480})_{sample} - [950/1{,}000 \times (A_{480})_{blank}]}{[1{,}050/1{,}000 \times (A_{480})_{standard}] - (A_{480})_{sample}} \times 5 \times n$$

$$(\text{mg/dL}) = \frac{(A_{480})_{sample} - [0.95 \times (A_{480})_{blank}]}{[1.05 \times (A_{480})_{standard}] - (A_{480})_{sample}} \times 5 \times n$$

n = dilution factor
$(A_{480})_{sample}$ = vale of sample (endogenous indican)
$(A_{480})_{blank}$ = value for the sample blank
$(A_{480})_{standard}$ = value for total indican (endogenous plus 5 mg/dL spiked indican)

Note: If calculated indican value is higher than 20 mg/dL, dilute sample in water and repeat assay. Multiply the results by the dilution factor (n).

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Presence of interfering substance in the sample	If possible, dilute sample further
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Samples measured at incorrect wavelength	Check the equipment and filter settings

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