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ab272529
Indican Assay Kit

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Indican Assay Kit datasheet:
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For quantitative determination of Indican activity and evaluation of drug effects on its metabolism.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

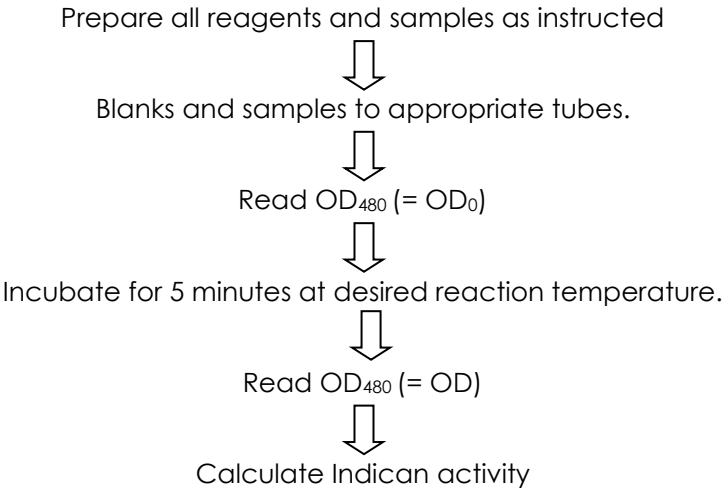
1. Overview

ab272529 is 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.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 12 months from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	20 mL	-20°C
Reagent B	1.5 mL	-20°C
Standard	1.5 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipetting devices
- Centrifuge tubes
- 96-well clear plate with flat bottom
- Standard microplate reader - capable of reading absorbance at 480 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

– Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 100 assays.

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

All reagents are supplied ready to use.

10. Sample Preparation

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.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to the desired reaction temperature (e.g. 25°C or 37°C) prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

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.

Component	Working Reagent mL	Standard mg/dL
Reagent A 1-20% hydrochloric acid	20	1.5 mL 30 mg/dL
Reagent B	1.5	1.5 mL 30 mg/dL

Δ Note: It is recommended that the Working Reagents be prepared fresh.

Reaction:

11.1 Transfer 50 µL urine samples into separate wells of the 96-well plate.

11.2 Add 140 µL Reagent A to each assay well. Tap plate to mix. Measure OD480nm (ODO) on a plate reader.

11.3 Add 10 µL Reagent B to each assay well. Tap plate to mix. Incubate 5 min and read OD480nm (ODS)

11.4 Add 10 µL provided standard to each assay well. Tap plate to mix. Incubate 5 min and read OD480nm (ODSTD).

Cuvette assay Procedure

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

Transfer 250 µL urine samples into separate cuvettes

Add 700 µL Reagent A to all samples. Tap cuvette to mix. Measure OD480nm (ODO) on a spectrophotometer.

Add 50 µL Reagent B to all cuvettes. Tap cuvette to mix. Incubate 5 min and read OD480nm (ODS).
Add 50 µL provided standard. Tap cuvette to mix. Incubate 5 min and read OD480nm (ODSTD).

12. Calculations

12.1 ADH activity can then be calculated as follows:

$$\text{Indican activity} = \frac{OD_S - OD_O}{OD_{STD} - OD_S} \times 5 \times n \text{ (mg/ dL)}$$

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

$$\text{Indican activity} = \frac{ODS - 950/1000 \times ODo}{1050/1000 \times ODSTD - ODS} \times 5 \times n \text{ (mg/ dL)}$$

where ODO = Sample Blank
ODS = endogenous indicant
ODSTD = total indican (endogenous + 5 mg/dL spiked indican)
n = Dilution factor

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.

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

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**.

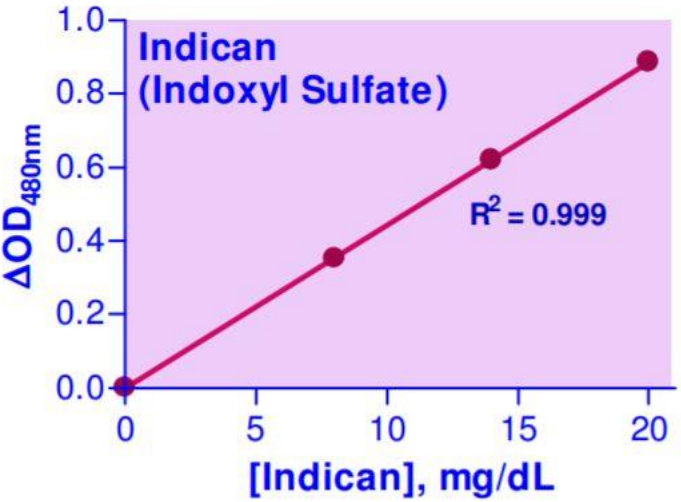


Figure 1. Example of Indican standard curve.

14. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)