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ab272540 Intestinal Permeability Assay Kit

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Intestinal Permeability Assay Kit datasheet:

www.abcam.com/ab272540

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For quantitative determination of determination of Intestinal Permeability (leaky gut syndrome) through measuring lactulose/mannitol ratio.

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

Table of Contents

1. Overview

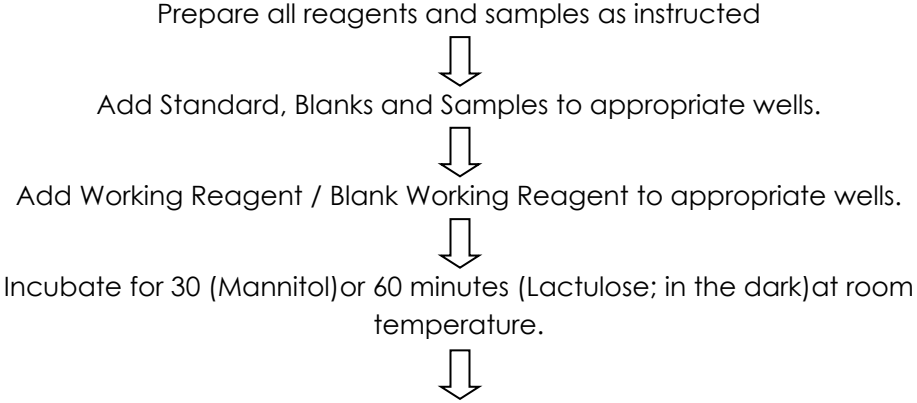
Intestinal Permeability Assay Kit (ab242540) is based on measuring the ratio of the absorption of two non-metabolized sugars through the intestines. Test subjects drink a prescribed amount of lactulose and mannitol and the % absorption of these sugars is determined by the amount of excreted lactulose and mannitol measured during the first 6 hours after ingestion. The degree of intestinal permeability is reflected by the ratio of the % absorption of lactulose to % absorption of mannitol. An increase in this ratio indicates increased intestinal permeability since lactulose is only absorbed though intercellular spaces. Lactulose and mannitol are measured in separate assays using the included Lactulose Assay Kit and Mannitol Assay Kit, respectively.

Simple and convenient: Both assays require addition of single working reagent and can be completed within 60 minutes. Both assays are performed at room temperature. No 37°C heater is needed.

High-throughput: Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Detection Limit: Mannitol - 7 to 3000 µM;
Lactulose – 3 to 300 µM.

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.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- 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.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 6 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
Lactulose Standard	400 µL	-20°C
Lactulose Assay Buffer	6 mL	-20°C
Lactulose Enzyme A	1 vial	-20°C
Lactulose Enzyme B	120 µL	-20°C
Lactulose Enzyme Buffer	150 µL	-20°C
Lactulose PMS Solution	1.5 mL	-20°C
Mannitol Standard	0.5 mL	-20°C
Mannitol Assay Buffer	10 mL	-20°C
Mannitol Enzyme	120 µL	-20°C

7. Materials Required, Not Supplied

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

- Distilled H_2O
- Pipettes
- 1.5 mL tubes
- 1.5 mL centrifuge
- Heat block or water bath
- 96-well clear plate with flat bottom
- Standard microplate reader - capable of reading at 520-600nm (peak absorbance is at 565 nm).
- Lactulose
- Mannitol

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.
- Remaining reagents are supplied ready to use.

Lactulose Enzyme A reconstitution:

9.1.1 Reconstitute Lactulose Enzyme A by adding 120 µL Enzyme Buffer to the Enzyme tube. Make sure enzyme is fully dissolved by pipetting up and down.

9.1.2 Store reconstituted enzyme at -20°C and use within 1 month.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

For Lactulose Assay:

10.1.1 Prepare 500 µL 300 µM Premix by mixing 10 µL Lactulose Standard (15 mM) and 490 µL distilled water.

10.1.2 Dilute standards in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix (µL)	H_2O (µL)	Lactulose (µM)
1	100	0	300
2	60	40	180
3	30	70	90
4	0	100	0

For Mannitol Assay:

10.1.3 Prepare 200 µL 3 mM Premix by mixing 30 µL Mannitol Standard (15 mM) and 470 µL distilled water.

10.1.4 Dilute standards in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix (µL)	H_2O (µL)	Mannitol (µM)
1	100	0	3000
2	60	40	1800
3	30	70	900
4	0	100	0

11. Sample Preparation

11.1 Patient Preparation:

11.1.1 Patients should fast overnight (or 8 hours minimum).

11.1.2 After emptying their bladder, patients should drink a 200 mL solution of 10 g lactulose and 5 g mannitol followed by 300 mL of water.

11.1.3 All urine should be collected for the next 6 hours after ingesting the sugar solution.

11.1.4 No food should be consumed for the 6 hours of urine collection, but an additional 200 mL of water should be taken 3 hours into the collection time.

11.2 Sample (Urine) Preparation:

11.2.1 The total volume of urine collected should be measured.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

For Lactulose Assay:

- Immediately prior to starting the reaction, as detailed in the table below.

12.1.1 Prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 50 µL Lactulose Assay Buffer, 14 µL Lactulose PMS Solution, 1 µL Lactulose Enzyme A and 1 µL Lactulose Enzyme B.

12.1.2 Prepare enough Blank Working Reagent (BWR) by mixing for each sample blank well, 50 µL Assay Buffer, 1 µL Enzyme A, and 14 µL PMS Solution (i.e. no Enzyme B)

Component	Working Reagent (µL/reaction)	Blank Working Reagent (µL/reaction)
Lactulose Assay Buffer	50	50
Lactulose PMS Solution	14	14
Lactulose Enzyme A	1	1
Lactulose Enzyme B	1	-

ΔNote: Do not expose Working Reagent / Blank Working Reagent to light for more than 10 minutes.

12.1.3 Add 40 µL of each standard (#1-4) to separate wells.

12.1.4 Add 40 µL of each sample into each of 2 separate wells: Sample well (OD_{SAMPLE}) and Blank Sample (OD_{BLANK}) well.

12.1.5 Add 60 µL of the WR to each Sample and Standard well.

12.1.6 Add 60 µL of the Blank WR to Blank Sample wells.

12.1.7 Tap plate to mix briefly and thoroughly.

12.1.8 Incubate for 60 min at room temperature in the dark.

12.1.9 Read OD at 565 nm (520-600 nm).

Δ Note: Use 96-well clear, flat-bottom plates.

For Mannitol Assay:

– Immediately prior to starting the reaction, as detailed in the table below.

12.1.10 Prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 85 µL Mannitol Assay Buffer and 1 µL Mannitol Enzyme.

Component	Working Reagent (µL/reaction)	Blank Working Reagent (µL/reaction)
Mannitol Assay Buffer	85	85
Mannitol Enzyme B	1	-

12.1.11 Add 20 µL of each standard (#1-4) to separate wells.

12.1.12 Add 20 µL of each sample into each of separate wells: Sample wells ($OD_{SAMPLE)}$ and Blank Sample (OD_{BLANK}) well.

12.1.13 Add 80 µL of the WR to each Sample and Standard well.

12.1.14 Add 80 µL of the Blank WR to Blank Sample wells.

12.1.15 Tap plate to mix briefly and thoroughly.

12.1.16 Incubate for 30 min at room temperature.

12.1.17 Read OD at 565 nm (520-600 nm).

Δ Note: Use 96-well clear, flat-bottom plates.

13. Calculations

Lactulose concentration:

13.1.1 Subtract Standard #4 (dH_2O) OD value from the remaining Standard OD values and plot the ΔOD against standard concentrations.

13.1.2 Determine the slope.

13.1.3 The Lactulose concentration of the Sample is calculated as follows:

$$[Lactulose]\ in\ \mu M\ = \frac{OD_{Sample} - OD_{Blank}}{Slope}\ X\ n$$

OD_{Sample} = OD value of the Sample
OD_{Blank} = OD value of Blank Sample
Slope = Standard curve slope (μM^{-1})
n is the dilution factor.

ΔNote: I the sample OD value is higher than OD for the 300 µM lactulose standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor

Conversions: 1 mM lactulose equals 34.2 mg/dL, or 342 ppm.

Mannitol concentration:

13.1.4 Subtract Standard #4 (dH_2O) OD value from the remaining Standard OD values and plot the ΔOD against standard concentrations.

13.1.5 Determine the slope.

13.1.6 The Mannitol concentration of the Sample is calculated as follows:

$$[Mannitol]\ in\ mM\ = \frac{OD_{Sample} - OD_{Blank}}{Slope}\ X\ n$$

OD_{Sample} = OD value of the Sample
OD_{Blank} = OD value of Blank Sample
Slope = Standard curve slope (mM^{-1})
n is the dilution factor

ΔNote: if the sample OD value is higher than OD for the 3 mM mannitol standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM D-mannitol equals 18.2 mg/dL, or 182 ppm.

Lactulose Mannitol Ratio:

13.1.7 The % Absorption of lactulose and mannitol are calculated as follows:

$$Lactulose\ Absorption\ = \frac{[Lactulose](\mu M)\ x\ 0.342\ \mu g/mL\ x\ Urine\ Vol\ (mL)}{10\ x\ 10^6\ \mu g}\ x\ 100\%$$

$$Mannitol\ Absorption\ = \frac{[Mannitol](mM)\ x\ 0.182\ mg/mL\ x\ Urine\ Vol\ (mL)}{5000\ mg}\ x\ 100\%$$

– Urine Vol is the total volume of urine collected for 6 hours.

13.1.8 The Lactulose Mannitol Ratio can then be calculated as follows:

$$Lactulose\ Mannitol\ Ratio = \frac{Lactulose\ Absorption}{Mannitol\ Absorption}$$

– For normal samples the ratio should be < 0.05.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

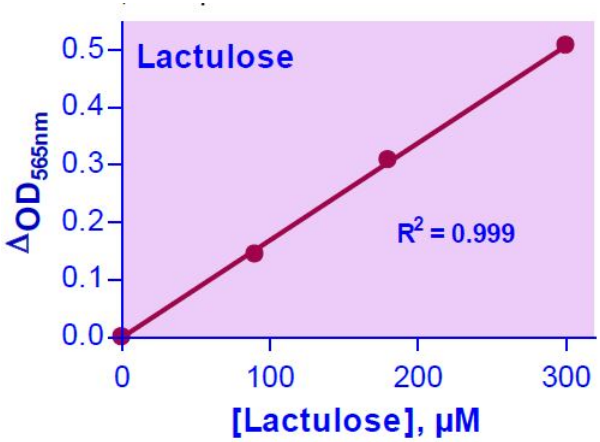


Figure 1. Example of Lactulose standard curve.

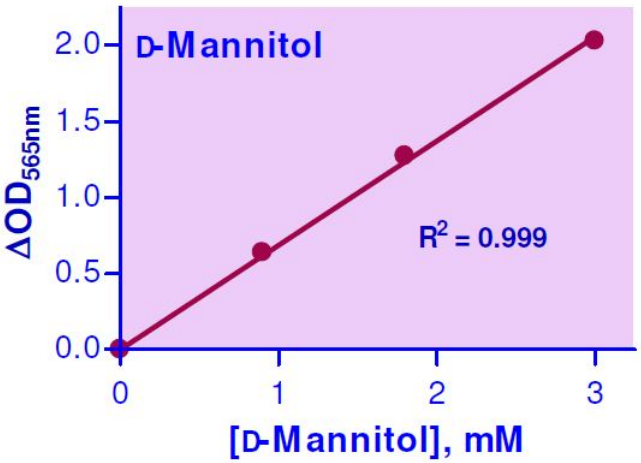


Figure 2. Example of Lactulose standard curve.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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