CREATIVE BIOMART®
Assay Kit

Intestinal Permeability Assay Kit

Product Information

Common Name

Permeability

Cat.No. Kit-2505

Product Overview

Intestinal permeability assay kit is based on measuring the ratio of the absorption of two non-metabolized sugars to 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.

Description

INTESTINAL PERMEABILITY is a phenomenon of the gut wall in which leakage of molecules and ions below 0.4 nm occurs from the gut lumen into blood circulation. This paracellular leakage occurs through tight junctions between epithelial cells. Elevated paracellular leakage has been implicated in many disorders including type 1 and type 2 diabetes, obesity, inflammatory bowel disease, celiac disease, Parkinson's disease and cancer.

Applications

Determination of intestinal permeability (leaky gut syndrome) through measuring lactulose/mannitol ratio.

Stability

Shelf life: 6 months after receipt.

Storage

The kit is shipped on ice. Store all components at -20°C upon receiving.

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Kit Components

LACTULOSE ASSAY KIT
Assay Buffer: 6 mL
Standard: 400 µL 15 mM Lactulose
Enzyme A: Dried
Enzyme B: 120 µL
Enzyme Buffer: 150 µL
PMS Solution: 1.5 mL

MANNITOL ASSAY KIT
Assay Buffer: 10 mL
Standard: 0.5 mL 20 mM D-Mannitol
Enzyme: 120 µL

Materials Required but Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates and plate or cuvette reader. Lactulose is available as osmotic laxatives in many drug stores (GoodRx, Rite-Aid, CVS, Walmart, Sams Club, Target etc). USP grade D-mannitol can be purchased from NuSci Institute & Corp., Avantor Performance Materials (J.T. Baker, cat# 2553-01), Sigma-Aldrich (cat# M8429). Use your own discretion when choosing appropriate materials for this test.
Procedure for preparation of the Lactulose/Mannitol solution: using the GoodRx Lactulose Solution (10g/15mL) as an example, mix 15 mL GoodRx lactulose and 5 g mannitol in a volumetric cylinder. Add water to the 200 mL mark.

Technical Notes

Reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents.

Features & Benefits

Simple and convenient. Both assays require addition of single working reagent and can be

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completed within 60 minutes. Both assays are performed at room temperature. No 37°C heater is needed.
High-throughput. 'Add-mix-read' type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Preparation

Patient Preparation: Patients should fast overnight (or 8 hours minimum). 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. All urine should be collected for the next 6 hours after ingesting the sugar solution. 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.

Sample Preparation: total volume of urine collected should be measured.

Assay Protocol

1. Lactulose Measurement.

Reagent Preparation: Reconstitute Enzyme A by adding 120 µL Enzyme Buffer to the Enzyme tube. Make sure enzyme is fully dissolved by pipetting up and down. Store reconstituted enzyme at -20°C and use within 1 month.

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor n.
Solid samples can be homogenized in distilled water followed by filtration or centrifugation (e.g. 5-10 min 14,000 rpm). Beverage samples can be assayed directly. Check the pH of the sample and neutralize if necessary. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor n = 1.36)
Biological fluid samples (e.g. urine) can be assayed directly. Appropriate dilution in distilled water may be required.

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Procedure using 96-well plate
1. Standards. Prepare 500 µL 300 µM Premix by mixing 10 µL of the Standard (15 mM) and 490 µL distilled water. Dilute standards in 1.5 mL centrifuge tubes as described in the Table.
No Premix + H2O Lactulose (µM)
1 100 µL + 0 µL 300
2 60 µL + 40 µL 180
3 30 µL + 70 µL 90
4 0 µL + 100 µL 0
2. Transfer 40 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 40 µL of each sample into two separate wells, one serving as a sample blank well (ODBLANK) and one as a sample well (ODSAMPLE).
3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 50 µL Assay Buffer, 14 µL PMS Solution, 1 µL Enzyme A, and 1 µL Enzyme B. Prepare 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).
Keep WR and BWR protected from light. Add 60 µL WR to the four Standards and the Sample Wells. Add 60 µL BWR to the Sample Blank Wells. Do not expose Working Reagent to light for more than 10 minutes. Incubate 60 min at room temperature in the dark.
4. Read optical density at 565 nm (520-600 nm).

CALCULATION
Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the lactulose concentration of Sample,
[Lactulose] = (ODSAMPLE – ODBLANK)/Slope (mM-1) * n (uM)
ODSAMPLE and ODBLANK are optical density readings of the Sample and Sample Blank, respectively. n is the sample dilution factor.
Note: if 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.

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2. Mannitol Measurement.

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor n.
Solid samples can be homogenized in distilled water followed by filtration or centrifugation (e.g. 5-10 min 14,000 rpm).
Beverage samples can be assayed directly. Check the pH of the sample and adjust to 8-9 with NaOH or HCl if necessary. Samples containing carbon dioxide should be degassed by gentle stirring prior assay.
Biological fluid samples (e.g. urine & serum) can be assayed directly. Appropriate dilution in distilled water may be required.

Procedure using 96-well plate
1. Standards. Prepare 200 µL 3 mM Premix by mixing 30 µL of the Standard (20 mM) and 170 µL distilled water. Dilute standards in 1.5 mL centrifuge tubes as described in the Table.
No Premix + H2O D-Mannitol (mM)
1 100 µL + 0 µL 3.0
2 60 µL + 40 µL 1.8
3 30 µL + 70 µL 0.9
4 0 µL + 100 µL 0
2. Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 20 µL of each sample into two separate wells, one serving as a sample blank well (ODBLANK) and one as a sample well (ODSAMPLE).
3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 µL Assay Buffer plus 1 µL Enzyme.
Add 80 µL WR to the four Standards and the Sample Wells. Add 80 µL Assay Buffer (i.e. no Enzyme) to the Sample Blank Wells. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.
3. Read optical density at 565 nm (520-600 nm).

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CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the mannitol concentration of Sample,

[[D-Mannitol] = (ODSAMPLE – ODBLANK)/Slope (mM-1) * n (uM)

ODSAMPLE and ODBLANK are optical density readings of the Sample and Sample Blank, respectively. n is the sample dilution factor.

Note: 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.

Analysis

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

Lactulose Absorption = {[Lactulose](μM) × 0.342 μg/mL × Urine Vol (mL)} / (10 × 10^6 μg) × 100%

Mannitol Absorption = {[D-Mannitol](μM) × 0.182 mg/mL × Urine Vol (mL)} / 5000 mg × 100%

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

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

Lactulose/Mannitol Ratio = Lactulose Absorption / Mannitol Absorption

For normal samples the ratio should be < 0.05.

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