

EnzyChrom™ Intestinal Permeability Assay Kit (EIPM-100)

Quantitative Colorimetric Intestinal Permeability Determination

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.

BioAssay Systems' 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 through intercellular spaces. Lactulose and mannitol are measured in separate assays using the included EnzyChrom™ Lactulose Assay Kit and Mannitol Assay Kit, respectively.

KEY FEATURES

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.

APPLICATIONS

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

KIT CONTENTS

ENZYCHROM™ LACTULOSE ASSAY KIT (ELTL)

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

ENZYCHROM™ MANNITOL ASSAY KIT (EMNT)

Assay Buffer: 10 mL **Standard:** 0.5 mL 20 mM D-Mannitol
Enzyme: 120 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: 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. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

Procedure using 96-well plate

1. **Lactulose Measurement.** Follow protocol given with EnzyChrom™ Lactulose Kit (ELTL).
2. **Mannitol Measurement.** Follow protocol given with EnzyChrom™ Mannitol Kit (EMNT).

CALCULATION

Compute the lactulose concentration in µM as directed in the ELTL protocol.

Compute the mannitol concentration in mM as directed in the EMNT protocol. The % Absorption of lactulose and mannitol are calculated as follows:

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

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

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

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

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

For normal samples the ratio should be < 0.05.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), 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.

LITERATURE

1. Sequeira, IR, et al (2014). Standardising the Lactulose Mannitol Test of Gut Permeability to Minimise Error and Promote Comparability. PLOS One 9:e99256.
2. Barboza Jr., MS, et al (1999). Measurement of intestinal permeability using mannitol and lactulose in children with diarrheal diseases. Braz J Med Res 32: 1499-1504.
3. Camilleri, M, et al (2010). Understanding Measurements of Intestinal Permeability in Healthy Humans with Urine Lactulose and Mannitol Excretion. Neurogastroenterol Motil 22: e15-e26.