AssayGenie

TECHNICAL MANUAL

PicoSense Dual-Sugar Intestinal Permeability Assay Kit

- **SKU CODE:** ARMA00352
- **SIZE:** 100 Mannitol + 100 Lactulose Tests
- **DETECTION PRINCIPLE:** Fluorometric
- **RUO:** Research-Use-Only

Revised: 08/26

PicoSense Dual-Sugar Intestinal Permeability Assay Kit

Please read entire manual carefully before starting experiment.

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1. Intended Use

The PicoSense Dual-Sugar Intestinal Permeability Assay is a non-invasive test used to evaluate the integrity of the gastrointestinal mucosal barrier. After a solution containing two non-metabolizable sugars — lactulose and mannitol — is ingested, these sugars pass through the gut wall via different routes. Mannitol is readily absorbed transcellularly, acting as a marker for total absorptive surface area. In contrast, lactulose is mostly excluded by healthy tight junctions but can slip through the paracellular pathways if the mucosal barrier is compromised (colloquially termed "leaky gut").

Following ingestion, urine or blood is collected over several hours. The concentrations of the recovered sugars are then measured and the ratio determined. Because absolute absorption rates vary by individual, the assay relies on calculating the specific ratio of the disaccharide to the monosaccharide excreted or absorbed. An elevated ratio indicates increased intestinal permeability, allowing macromolecules to leak into the bloodstream. This makes the assay highly valuable for investigating conditions like Crohn's disease, irritable bowel syndrome, and food sensitivities.

This kit contains sufficient materials to make 100 determinations of each sugar along with standard curves for each.

This manual must be read in its entirety before using this product. **For Research Use Only. Not to be used for diagnostic or therapeutic purposes.**

2. Principle of the Assay

Lactulose in samples is hydrolysed to fructose and galactose. The galactose is oxidised by galactose dehydrogenase, forming NADH. Mannitol in samples is oxidised by mannitol dehydrogenase, also forming NADH. In both cases the NADH is used to convert resazurin to resorufin, with a large increase in fluorescence.

3. Materials Provided

Component	Quantity	Cap / Label Colour
Sample Clean-up Mix	Lyophilised	NM
Lactulose Buffer	25 ml	WM
Mannitol Buffer	25 ml	Amber WM
β-Galactosidase	Lyophilised	Blue
Mannitol Enzyme Mix	Lyophilised	Red
Lactulose Enzyme Mix	Lyophilised	Green
Resazurin	600 µl	Blue/Amber
Enhancer	2.2 ml	Clear
Lactulose Standard	500 µl	Yellow
Mannitol Standard	500 µl	Orange

4. Storage and Handling

Store the unopened kit at -20°C. Bring all components to room temperature before use. Centrifuge all vials for a few seconds before opening.

- **Sample Clean-up Mix:** Add 10 ml of 50% glycerol to the bottle and invert several times to disperse and mix. Store at -20°C.
- **Lactulose Buffer, Mannitol Buffer:** Ready to use as supplied. Store at 4°C.
- **β-Galactosidase, Lactulose Enzyme Mix:** Reconstitute with 220 µl of Lactulose Buffer. Keep on ice while in use. Store at -20°C.
- **Mannitol Enzyme Mix:** Reconstitute with 220 µl of Mannitol Buffer. Keep on ice while in use. Store at -20°C.
- **Resazurin:** Ready to use as supplied. Comes as a 2 mM solution. Store at -20°C.
- **Enhancer:** Ready to use as supplied. Store at 4°C.
- **Lactulose Standard (80 µM), Mannitol Standard (80 µM):** Ready to use as supplied. Store at -20°C.

5. Other Supplies Required

- White or black 96-well microplates.
- Fluorescence microplate reader capable of Ex/Em = 535/587 nm.
- 50% glycerol for reconstitution of the Sample Clean-up Mix.
- Deionised water.
- Eppendorf-type snap-cap tubes.
- Bench-top microcentrifuge capable of ~16,000 × g.
- 37°C incubator or water bath.
- Vortex mixer, pipettes and pipette tips.

6. Assay Procedure

The assay is composed of two distinct parts, a mannitol determination and a lactulose determination.

A. Mannitol Determination

1. **Sample Preparation:** Urine is taken before ingestion of a lactulose/mannitol cocktail as a background control and collected for 6 hours after ingestion and pooled. 100 µl of each urine sample is placed in an Eppendorf-type snap-cap tube and 100 µl of Sample Clean-up Mix is added. Vortex briefly to mix and place at 37°C for 30 minutes to scavenge any glucose or fructose which may be present. Centrifuge at top speed in a table-top centrifuge (~16,000 × g) for 5 minutes and transfer the clear supernatant to a fresh tube without disturbing the pellet.
2. **Mannitol Standard Curve:** Transfer 0, 5, 10, 15, 20 and 25 µl of the 80 µM Mannitol Standard to a series of wells in a white or black 96-well plate, giving 0, 0.4, 0.8, 1.2, 1.6 and 2 nmol of Mannitol, respectively. Adjust all standards to 25 µl with Mannitol Buffer.
3. **Mannitol Samples:** Take 10 µl of each sample and add 115 µl of DI water. Transfer 5 µl of each diluted sample to wells in the 96-well plate, in duplicate. One of each pair

will be used as a background control well. Adjust all samples to 25 µl with Mannitol Buffer.

4. **Fluorescence Development:** Each sample, background control and standard well will require 60 µl of Mannitol Reaction Mix. Make sufficient material for the number of mannitol standards, samples and background control wells to be analysed, containing:

Reaction Mix	Volume
Assay Buffer	54 µl
Enzyme Mix	2 µl
Resazurin	2 µl
Enhancer	2 µl

Mix and add 60 µl of Reaction Mix to each well containing samples, background controls and Standards. Mix well. Incubate at 37°C for 30 minutes then add 15 µl of Enhancer.

5. **Measurement:** Measure fluorescence using Ex/Em = 535/587 nm. This can be done either during the reaction while monitoring the kinetics of the reaction, or as an endpoint after the 30 minute incubation and enhancement.

Typical Results – Mannitol:

Standard (pmol)	Initial Raw Values (RFU)	Enhanced Values (RFU)
0	1828	425
400	2229	507
800	2713	578
1200	3267	695
1600	3875	802
2000	4524	919

B. Lactulose Determination

1. **Lactulose Standard:** Transfer 0, 5, 10, 15, 20 and 25 µl of the 80 µM Lactulose Standard to a series of wells in a white or black 96-well plate, giving 0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmol of Lactulose, respectively. Adjust all standards to 25 µl with Lactulose Buffer.

2. **Lactulose Samples:** Transfer 25 µl of each sample to wells in the 96-well plate, in duplicate. One of each pair will function as a background control well.
3. **Hydrolysis Reaction:** Add 2 µl of β-Galactosidase and 3 µl of Lactulose Buffer to each well containing samples or Standards. Add 5 µl Buffer to the background control wells. Cover the plate and incubate at 37°C for 30 minutes.
4. **Fluorescence Development:** Each Lactulose sample, standard and background control well will require 60 µl of Lactulose Reaction Mix. Prepare a sufficient amount for the total number of lactulose determinations to be made, containing:

Reaction Mix	Volume
Assay Buffer	54 µl
Enzyme Mix	2 µl
Resazurin	2 µl
Enhancer	2 µl

Mix and add 60 µl of Reaction Mix to each well containing samples, background controls and Standards. Mix well. Incubate at 37°C for 30 minutes then add 15 µl of Enhancer.

5. **Measurement:** Measure fluorescence using Ex/Em = 535/587 nm. This can be done either during the reaction while monitoring the kinetics of the reaction, or as an endpoint after the 30 minute incubation and enhancement.

Typical Results – Lactulose:

Standard (pmol)	Initial Raw Values (RFU)	Enhanced Values (RFU)
0	930	180
400	2594	437
800	3812	608
1200	5565	850
1600	6620	971
2000	8576	1226

7. Calculation of Results

Subtract the 0 standard reading from all standards for both the Mannitol and Lactulose assays. Plot each standard curve and determine their corresponding slopes. For the Mannitol and Lactulose samples, subtract each background control well reading from its paired sample reading. Divide the corrected RFU by the slope of the standard curve to convert from RFU to picomoles of mannitol or lactulose in the well.

To convert to lactulose or mannitol in the original sample:

- **For Mannitol:** pmol/µl of original urine = pmol in the well / 5 µl × 500 (dilution factor)
- **For Lactulose:** pmol/µl of original urine = pmol in the well / 25 µl × 8

Lactulose/Mannitol Ratio = pmol Lactulose / pmol Mannitol

8. Important Notes

- This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
- Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
- Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
- If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
- If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
- Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We

advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.