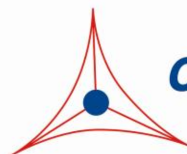
Product Manual

D-Mannitol Assay Kit

Catalog Number

MET-5218 200 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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Introduction

D-Mannitol is a sugar alcohol used as a low-calorie option to sweeten certain foods since it is not absorbed well by the body. It is also used medically to decrease ocular pressure in glaucoma or to lower abnormally high intracranial pressure. D-Mannitol is also used to treat some forms of kidney failure to improve toxin elimination, and to alleviate the buildup of fluid in the body. Infusions of D-Mannitol into the artery can disrupt tight junctions and open the blood brain barrier. This sugar alcohol is approved in Europe to treat cystic fibrosis in adults.

Cell Biolabs' D-Mannitol Assay Kit is a sensitive, quantitative colorimetric assay for D-Mannitol. The provided reagents are sufficient for the evaluation of 200 assays* including blanks, D-Mannitol standards, background controls and unknown samples. The kit has a detection sensitivity limit of 20 µM D-Mannitol.

Note: Each sample replicate requires 2 assays, one treated with mannitol dehydrogenase (+MDH) and one without (-MDH). D-Mannitol levels are calculated from the difference in OD readings from the 2 wells.

Assay Principle

The unknown samples or D-Mannitol standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and Mannitol Dehydrogenase (MDH). During a brief incubation the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of D-Mannitol in the unknown samples is determined by comparison with a predetermined D-Mannitol standard curve.

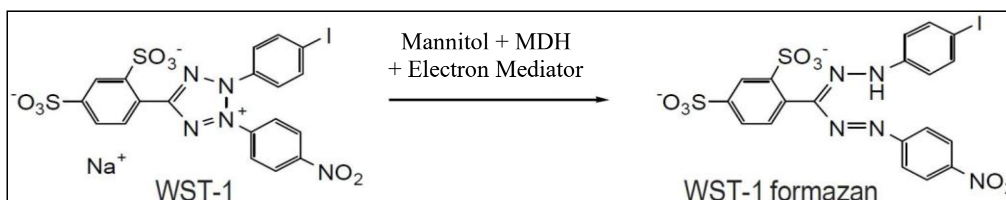

Figure 1. Assay Principle.

Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5056: Branched Chain Amino Acid Assay Kit (Colorimetric)
3. MET-5125: Pyruvate Assay Kit (Colorimetric)
4. MET-5070: Glycine Assay Kit (Fluorometric)
5. STA-680: Glucose Assay Kit (Colorimetric)

Kit Components (shipped on blue ice)

1. 10X Colorimetric Probe (Part No. 50801C): Two 2 mL amber vials
2. D-Mannitol Standard (Part No. 52181C): One 100 µL vial at 100 mM
3. 10X Assay Buffer (Part No. 52182B): One 30 mL bottle
4. 50X NAD+ (Part No. 50803D): One 800 µL vial
5. Mannitol Dehydrogenase (50X) (Part No. 52183D): One 800 µL vial

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate

Storage

Upon receipt, store the 10X Assay Buffer at 4°C. Store 50X NAD+ and Mannitol Dehydrogenase (50X) at -80ºC. Store all remaining components at -20ºC. The 10X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Dilute the 10X Colorimetric Probe, the Mannitol Dehydrogenase (50X) and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, 80 µL of Mannitol Dehydrogenase (50X), and 80 µL of 50X NAD+ to 3.44 mL of 1X Assay Buffer.

  Note: Scale down the described example appropriately and prepare only enough for immediate use.
- Control Mix: Dilute both the 10X Colorimetric Probe and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 400 µL of 10X Colorimetric Probe, and 80 µL of 50X NAD+ to 3.52 mL of 1X Assay Buffer.

  Note: Scale down the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Mannitol standard curve in the same non-conditioned media.

- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in deionized water or PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in deionized water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.
- Food samples: Homogenize 200 mg of food sample in 800 µL of deionized water or PBS. Centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.

Preparation of Standard Curve

Freshly prepare a dilution series of D-Mannitol Standards according to Table 1.

Standard Tubes	100 mM D-Mannitol Standard (µL)	Deionized Water (µL)	D-Mannitol (µM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 1. Preparation of D-Mannitol Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with MDH (Reaction Mix) and one without the enzyme (Control Mix) to account for endogenous sample background.
2. Add 50 µL of each sample (D-Mannitol standard or unknown) into wells of a 96 well plate.
3. Add 200 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 200 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at room temperature for 60 minutes on an orbital shaker.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical D-Mannitol Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

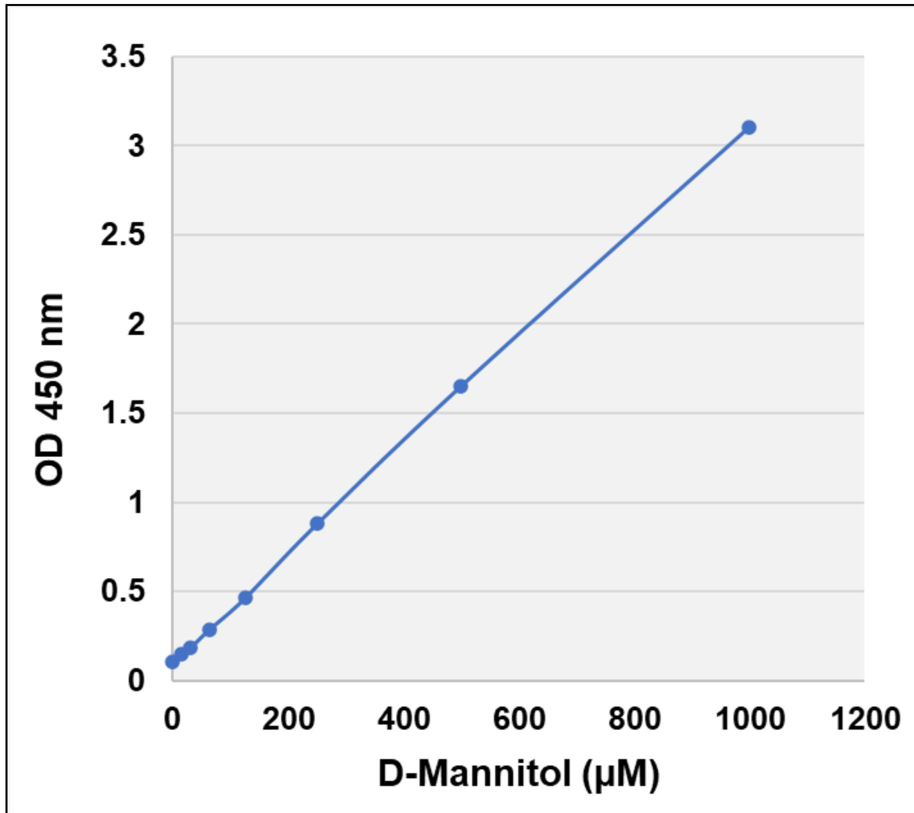

Figure 2. D-Mannitol Standard Curve.

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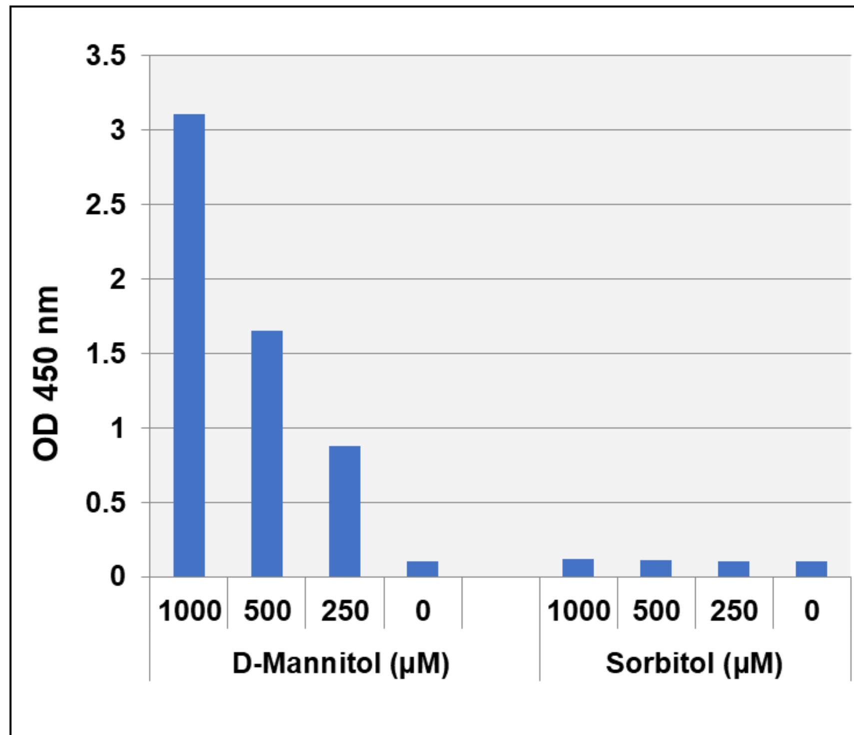

Figure 3. Specificity of D-Mannitol Assay.

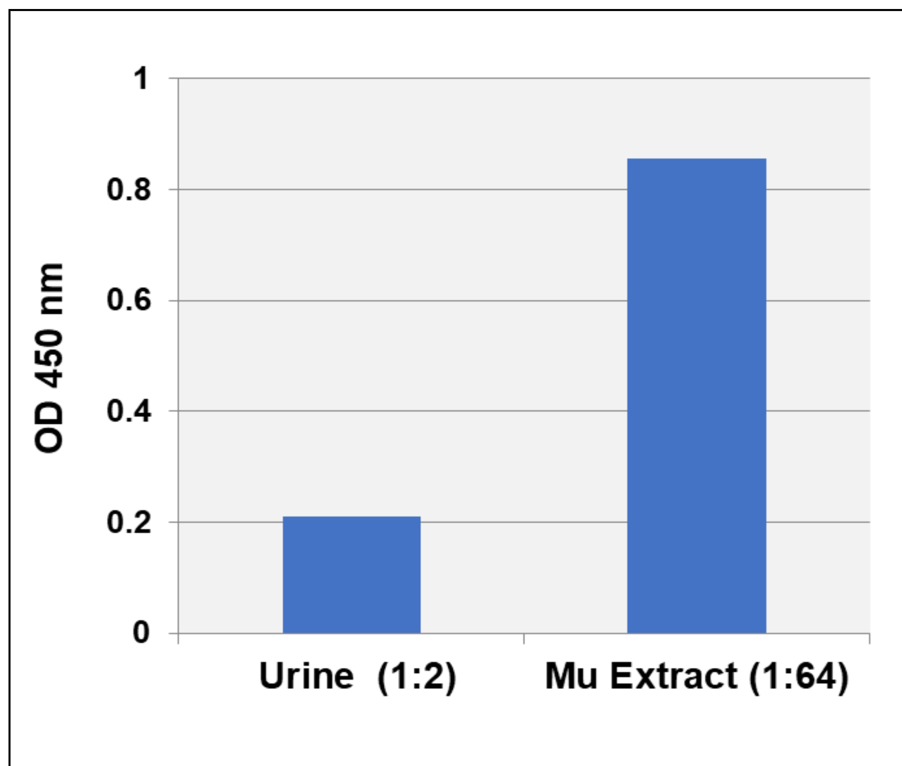

Figure 4. Detection of D-Mannitol in Human Urine and White Mushroom Extract. Human urine (Urine) was diluted 2-fold into deionized water and white mushroom extract (Mu Extract) was diluted 64-fold into deionized water before performing the Mannitol assay according to the kit protocol.

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Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without MDH (-MDH) from the sample well values containing enzyme (+MDH) to obtain the difference. The absorbance difference is due to the enzyme MDH activity:

$$\Delta A = A_{(+MDH)} - A_{(-MDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of D-Mannitol present in the sample. Only use values within the range of the standard curve.

References

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