Lactose Microplate Assay Kit

User Manual

Catalog # CAK1260

(Version 1.3A)

Detection and Quantification of Glucose Content in Serum, Plasma, Urine, Saliva, Milk, Tissue extracts, Cell lysate, Cell culture and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lactose ($C_{12}H_{22}O_{11}$), also called milk sugar, is a disaccharide that consists of β-D-galactose and α/β-D-glucose through a β1-4 glycosidic linkage. Lactose is the major sugar and makes up 2-8% of milk.

Lactose Microplate Assay Kit provides a simple and direct procedure for measuring lactose levels in a variety of samples. Lactose is hydrolysed by lactase (β-galactosidase), released galactose and glucose. Then glucose can be hydrolysed by glucose oxidase. The enzyme catalysed reaction product H_2O_2 can be measured at a colorimetric readout at 505 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	30 ml x 1	4 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	Powder x 2	-20 °C
Reaction Buffer	15 ml x 1	4 °C
Dye Reagent	Powder x 2	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme I: add 1 ml Reaction Buffer to dissolve before use.

Enzyme II: add 1 ml Reaction Buffer for each tube to dissolve before use.

Dye Reagent: add 10 ml distilled water for each tube to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.5 ml into 0.5 ml distilled water, the concentration will be 20 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 µl distilled water for 5×10^6 cells or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 250 µl Assay Buffer I mix, and 250 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5 ml distilled water, transfer it into the centrifuge tube; then add 250 µl Assay Buffer I mix, and 250 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Serum and plasma samples can be assayed directly.

Milk samples should be cleared by mixing 500 µl sample with 250 µl Assay Buffer I and 250 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection (dilution factor n = 2).

V. ASSAY PROCEDURE

Warm the Reaction Buffer to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl	60 µl
Sample	20 µl	20 µl	--	--
Standard	--	--	20 µl	--
Distilled water	--	10 µl	--	20 µl
Enzyme I	10 µl	--	10 µl	10 µl
Mix, cover the plate adhesive strip, put it in the oven, incubate at 37 °C for 30 minutes.				
Enzyme II	10 µl	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, cover the plate adhesive strip, put it in the oven, incubate at 37 °C for 20 minutes, record absorbance measured at 505 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{Lactose } (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the quantity of cells or bacteria

$$\text{Lactose } (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the volume of sample

$$\text{Lactose } (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) \times n$$

$C_{Standard}$: the standard concentration, 20 mmol/L = 20 μmol/ml;

$V_{Standard}$: the volume of standard, 20 μl = 0.02 ml;

V_{Sample}: the volume of sample, 20 μl = 0.02 ml;

W: the weight of sample, g;

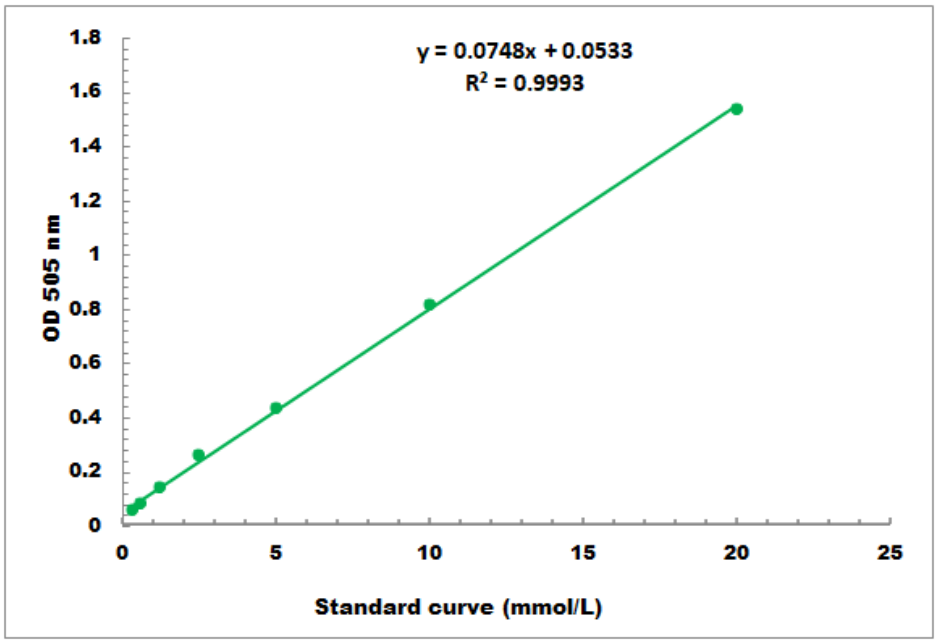
N: the quantity of cell or bacteria, N × 10^4;

V_{Assay}: the volume of distilled water, Assay Buffer I and Assay Buffer II, 1 ml;

n: dilution factor.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.2 mmol/L - 20 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES