Galactose Microplate Assay Kit

User Manual

Catalog # CAK1259

(Version 1.4B)

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

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

I. INTRODUCTION

Galactose ($C_6H_{12}O_6$) is a monosaccharide that is found in dairy products, sugar beets, gums and mucilages. It is also synthesized in mammals, where it forms part of glycolipids and glycoproteins in several tissues. It forms the disaccharide lactose when combined with glucose.

Galactose Microplate Assay Kit provides a simple and direct procedure for measuring galactose levels in a variety of samples. In this reaction, galactose dehydrogenase may catalyze galactose and NAD to galactonic acid and NADH. The amount of NADH formed in this reaction is proportionate to the amount of galactose. The intensity of the product color, measured at 450 nm, is proportional to the Galactose concentration in the sample.

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
Reaction Buffer	10 ml x 1	4 °C
Coenzyme	Powder x 1	4 °C
Enzyme	50 µl x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Coenzyme: add 1 ml Reaction Buffer to dissolve before use.

Enzyme: add 1 ml Reaction Buffer to dilute before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, the concentration will be 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge

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	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Coenzyme	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, incubate at 37 °C for 30 minutes.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, measured at 450 nm immediately and record the absorbance.			

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

$$\text{Galactose (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the quantity of cells or bacteria

$$\text{Galactose (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the volume of sample

$$\text{Galactose (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 1 mmol/L = 1 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;

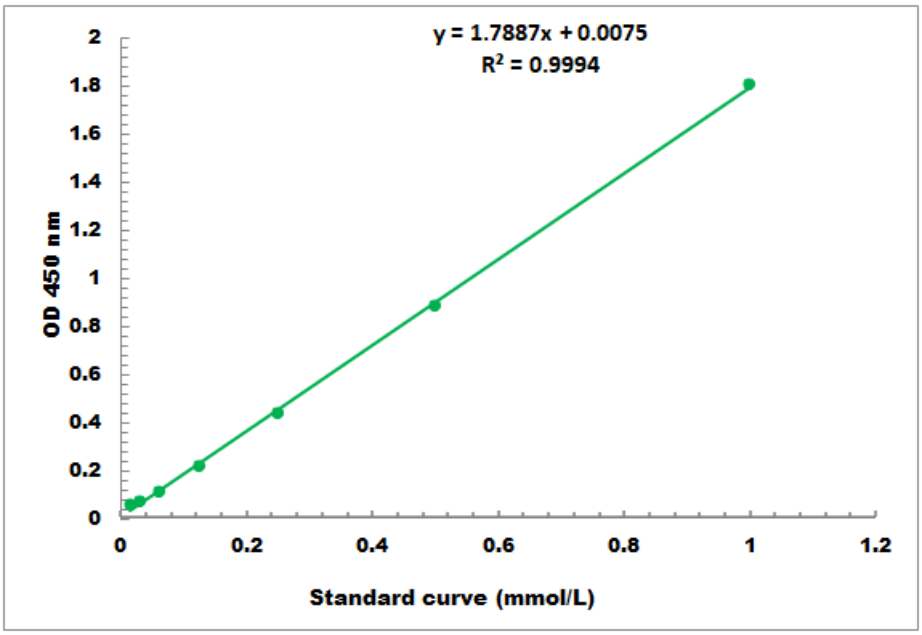
N: the quantity of cell or bacteria, N × 10^4;

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

n: dilution factor = 2.

VII. TYPICAL DATA

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



Detection Range: 0.01 mmol/L - 1 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