KAMIYA BIOMEDICAL COMPANY

Galactose Colorimetric Detection Kit

For the quantitative determination of galactose in serum, plasma, buffers and TCM

Cat. No. KT-724

For Research Use Only.

PRODUCT INFORMATION

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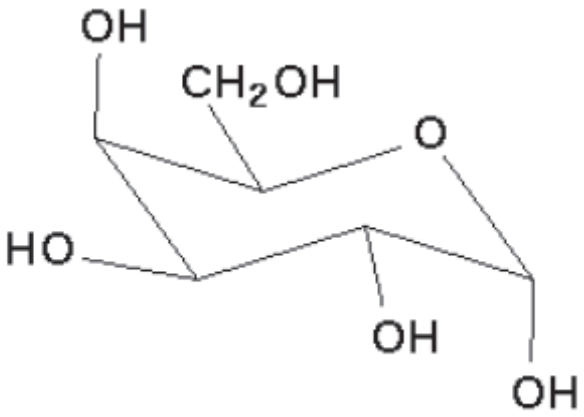
BACKGROUND

Galactose is a hexose sugar that differs from glucose only by the configuration of the hydroxyl group at the carbon-4 position. Present as an anomeric mixture of α-D-galactose and ß-D-galactose, this monosaccharide exists abundantly in milk, dairy products and many other food types such as fruits and vegetables. Absorption of galactose in humans is mediated by the Na+/glucose co-transporters SGLT1 and SGLT2 from food across the brush border membrane of the proximal jejunum and renal epithelium. Other sources of galactose include endogenous production and natural turnover of glycolipids and glycoproteins. Adult humans can produce up to 2 grams of galactose per day.



Galactose

Inside the cells, ß-D-galactose is epimerized to α-D-galactose through the action of a mutarotase. α-D-galactose is subsequently converted to galactose-1-phosphate (Gal-1-P) by the enzyme galactokinase. In the presence of galactose-1-phosphate uridylyltransferase, Gal-1-P reacts with UDP-glucose to form UDP-galactose and glucose-1-phosphate. Glucose-1-phosphate produced can enter the glycolytic pathway or react with UTP in the presence of UDP-glucose pyrophosphorylase to form a new molecule of UDP-glucose. This enzyme pathway comprises the evolutionarily conserved Leloir pathway of galactose metabolism. If the flow of galactose through the Leloir pathway is perturbed either due to congenital deficiency of any of the above-mentioned enzymes or an overwhelming presence of this hexose, toxicity syndromes (galactosemia) will be observed. Its cause as a defect in galactose metabolism was identified by a group led by Kalckar in 1956.

PRINCIPLE

The Galactose Colorimetric Detection Kit is designed to quantitatively measure galactose in a variety of samples. Please read the complete kit insert before performing this assay. A D-(+)-galactose calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Samples are mixed with the Colorimetric Substrate and horseradish peroxidase and the reaction initiated by addition of galactose oxidase. The reaction is incubated at room temperature for 30 minutes. The galactose oxidase reacts with galactose to produce hydrogen peroxide which, in the presence of HRP, reacts with the colorless Colorimetric Substrate to produce a pink-colored product which is read at 560 nm. Increasing levels of galactose cause a linear increase in color.

COMPONENTS

Clear 96 well Half Area Plates 2 Plates
Corning Costar Plate 3695.

Galactose Calibrator 90 µL
Galactose at 250 mg/dL in a special stabilizing solution.

Assay Buffer 50 mL
Assay buffer containing detergents and stabilizers.

Colorimetric Substrate 5 mL
A solution of the substrate in a special stabilizing buffer.

Horseradish Peroxidase Concentrate 60 µL
A 100X concentrated solution of HRP in a special stabilizing solution.

Galactose Oxidase 2 Vials
Freeze dried solution of Galactose Oxidase stored in a desiccator.

STORAGE

All components of this kit should be stored at 4℃ until the expiration date of the kit.

Once reconstituted, the Galactose Oxidase **must** be stored at -20℃.

OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25 µL.

96 well plate reader capable of reading at 560 nm (Acceptable Range 540-580 nm.). Set plate parameters for a 96-well Corning Costar 3695 plate.

Software for converting colorimetric intensity readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. **This product is not for Human Diagnostic Use.**

SAMPLE TYPES AND PREPARATION

Samples that need to be stored after collection should be stored at -70℃ or lower, preferably after being frozen in liquid nitrogen. Serum and plasma samples can be used after being diluted ≥ 1:15. This assay has been validated for buffer and media samples.

REAGENT PREPARATION

Horseradish Peroxidase (HRP) Preparation
Dilute the HRP Stock solution 1:100 with Assay Buffer using the table below:

HRP Dilution Table

	1/2 Plate	One Plate	Two Plates
HRP Stock	15 µL	30 µL	55 µL
Assay Buffer	1.485 mL	2.97 mL	5.445 mL
Total Volume	1.5 mL	3 mL	5.5 mL

Galactose Oxidase (GOD) Preparation

Allow the desiccator to warm to room temperature.
Add 3.125 mL of the Assay Buffer to a Galactose Oxidase vial and vortex thoroughly. Each vial contains enough GOD for one plate.

Unused prepared Galactose Oxidase solution should be stored at -20℃ after reconstitution.

Calibrator Preparation
Galactose Calibrators are prepared by labeling seven tubes as #1 through #6. Briefly vortex to mix the vial of Galactose Calibrator. Pipet 135 µL of Assay Buffer into tube #1. Pipet 75 µL of Assay Buffer into tubes #2 to #6. Carefully add 15 µL of the Galactose Calibrator to tube #1 and vortex completely. Take 75 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #6. The concentration of galactose in tubes 1 through 6 will be 25, 12.5, 6.25, 3.125, 1.56, and 0.781 mg/dL.

Use all Calibrators within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**135**	75	75	75	75	75
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**15**	75	75	75	75	75
Final Conc (mg/dL)	25	12.5	6.25	3.125	1.56	0.781

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. Set plate parameters for a 96-well Corning Costar 3695 plate.

1. Pipet 20 µL of diluted samples or calibrators into duplicate wells in the plate.

2. Pipet 20 µL of Assay Buffer into duplicate wells as the Zero calibrator.

3. Add 25 µL of the prepared HRP solution to each well using a repeater pipet.

4. Add 25 µL of the Colorimetric Substrate solution to each well using a repeater pipet.

4. Initiate the reaction by adding 25 µL of the prepared GOD solution to each well using a repeater pipet.

5. Incubate at room temperature for 30 minutes.

6. Read the plate at 560 nm (Acceptable Range 540-580 nm.).

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean ODs for the Zero wells. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

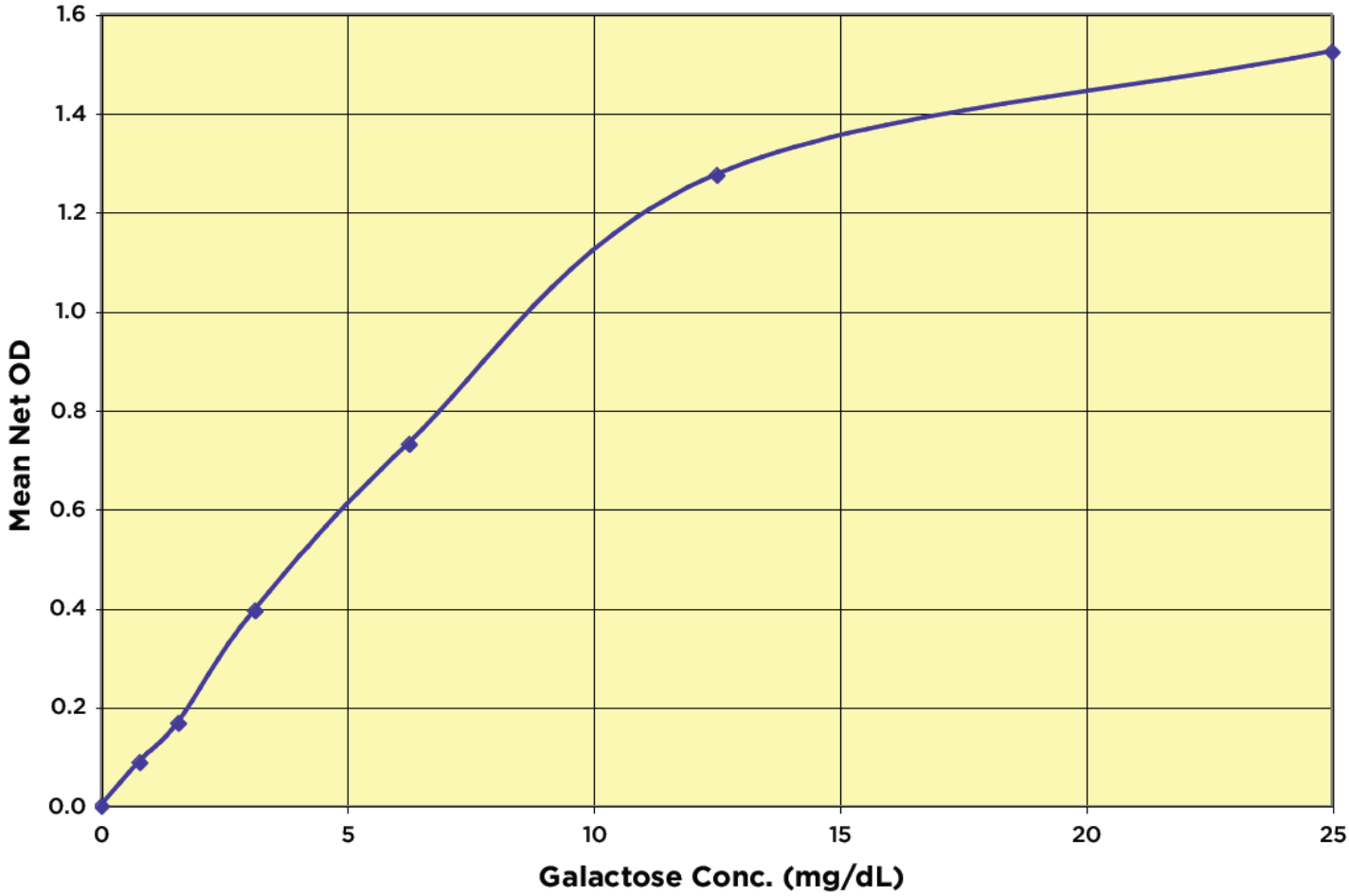
TYPICAL DATA

Sample	Mean OD	Net OD	Galactose Conc. (mg/dL)
Zero	0.099	0.000	0
Standard 1	1.623	1.524	25
Standard 2	1.374	1.275	12.5
Standard 3	0.830	0.731	6.25
Standard 4	0.494	0.395	3.125
Standard 5	0.267	0.168	1.56
Standard 6	0.187	0.088	0.781
Sample 1	1.122	1.023	9.04
Sample 2	0.454	0.355	2.97

Always run your own calibration curves for calculation of results. Do not use these data.

Conversion Factor: 100 mg/dL of Galactose is equivalent to 1 mg/mL or 5.55 mM.

Typical Calibration Curve



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VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and calibrator #6. The detection limit was determined at two (2) standard deviations from the zero along the calibration curve.

Sensitivity was determined as 0.493 mg/dL.

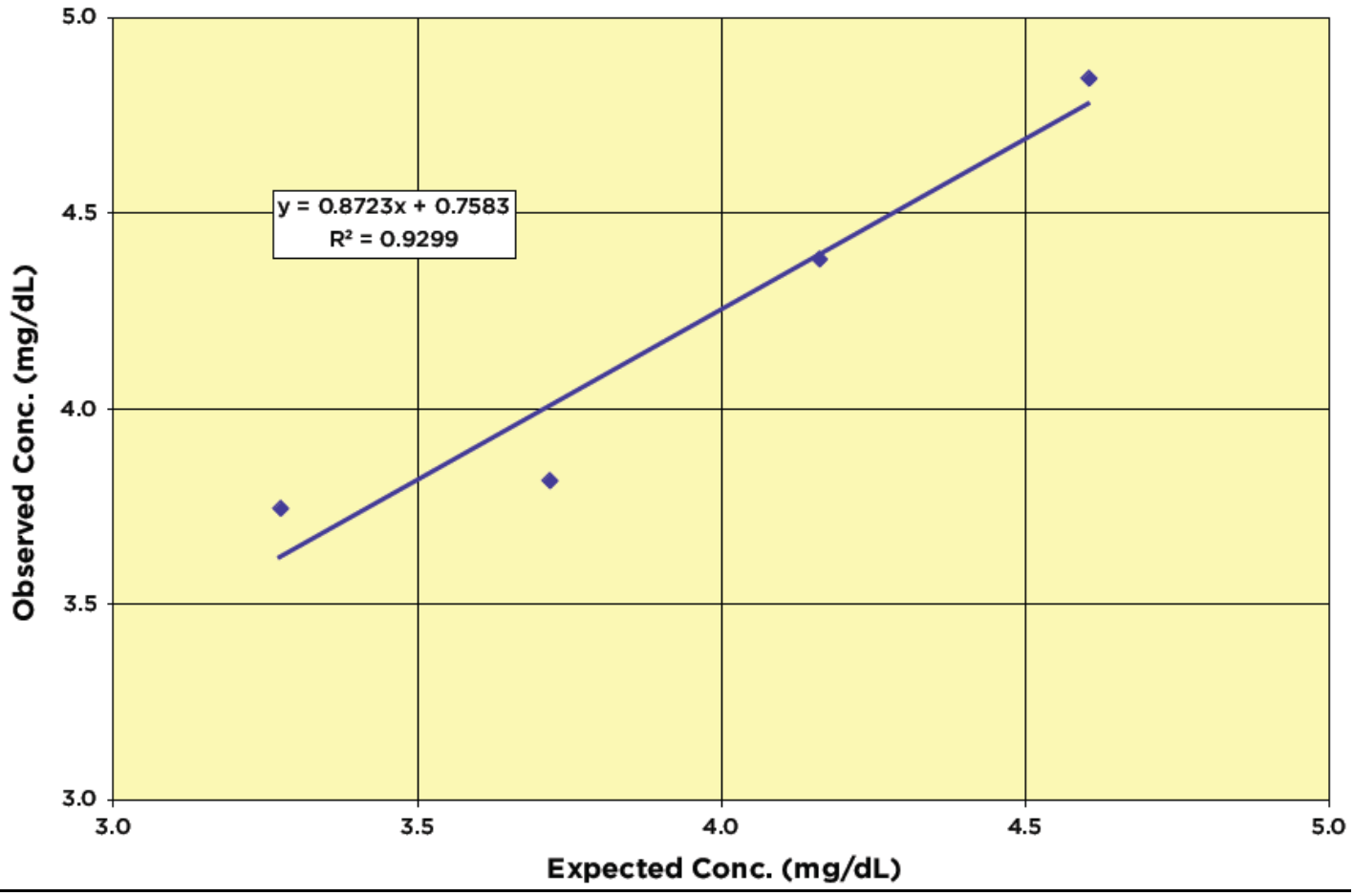
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.

The Limit of Detection was determined as 0.383 mg/dL.

Linearity

Linearity was determined in serum samples by taking two diluted samples with known galactose concentrations, one sample with a high galactose concentration of 5.05 mg/dL and one with a lower value of 2.83 mg/dL, mixing in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Sample	High Sample	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	3.74	3.28	114.3
60%	40%	3.82	3.72	102.6
40%	60%	4.38	4.16	105.2
20%	80%	4.84	4.61	105.2
			Mean Recovery	**106.8%**



Intra Assay Precision

Three diluted spiked samples were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	Galactose Conc. (mg/dL)	%CV
1	9.58	4.8
2	5.75	3.6
3	3.18	6.2

Inter Assay Precision

Three diluted spiked serum samples were run in duplicate in twenty assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	Galactose Conc. (mg/dL)	%CV
1	8.50	8.4
2	5.41	5.1
3	2.95	4.8

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com

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