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ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
MOST SENSITIVE

DetectX®

Galactose
Colorimetric Detection Kit

2 Plate Kit Catalog Number K042-H1

Species Independent

Sample Types Validated:

Serum, Plasma, Buffers and TCM

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.
Not for human diagnostic use.

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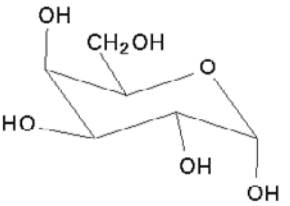
BACKGROUND

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 β-D-galactose, this monosaccharide exists abundantly in milk, dairy products and many other food types such as fruits and vegetables[1,2]. 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[3-6]. 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[7].



Galactose

Inside the cells, β-D-galactose is epimerized to α-D-galactose through the action of a mutarotase[8]. 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[9].

1. Acosta, P. B., & Gross, K. C. (1995). Hidden sources of galactose in the environment. *European Journal of Pediatrics*, 154(Suppl 2), S87–S92.
2. Berry, G. T., et al. (1993). The effect of dietary fruits and vegetables on urinary galactitol excretion in galactose-1-phosphate uridyltransferase deficiency. *Journal of Inherited Metabolic Disease*, 16(1), 91–100.
3. Martin, M. G., et al. (1996). Defects in Na+/glucose cotransporter (SGLT1) trafficking and function cause glucose-galactose malabsorption. *Nature Genetics*, 12, 216–220.
4. Wright, E. M., et al. (1992). The Na+/glucose cotransporter (SGLT1). *Acta Physiologica Scandinavica. Supplementum*, 607, 201–207.
5. Longo, N., & Elsas, L. J. (1998). Human glucose transporters. *Advances in Pediatrics*, 45, 293–313.
6. Elsas, L. J., et al. (1971). Autosomal recessive inheritance of renal glycosuria. *Metabolism*, 20(10), 968–975.
7. Berry, G. T., et al. (1995). Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia. *The Lancet*, 346(8982), 1073–1074.
8. Thoden, J. B., et al. (2004). Molecular structure of human galactose mutarotase. *Journal of Biological Chemistry*, 279, 23431–23437.
9. Isselbacher, K. J., et al. (1956). Congenital galactosemia, a single enzymatic block in galactose metabolism. *Science*, 123(3198), 635–636.

ASSAY PRINCIPLE

The DetectX® 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 standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are mixed with the Substrate and horseradish peroxidase and the reaction initiated by addition of galactose oxidase. The plate 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 Substrate to produce a pink-colored product to be read at 560 nm. Increasing levels of galactose cause a linear increase in color.

RELATED PRODUCTS

Kits	Catalog No.
Glucose Colorimetric Detection Kit	K039-H1
Glucose Fluorescent Detection Kit	K039-F1
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Urea Nitrogen (BUN) Detection Kit	K024-H1
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Clear Half Area 96 Well Plates
Corning Costar Plate 3695.
2 Plates — Catalog Number X018-2EA

Galactose Standard
Galactose at 250 mg/dL in a special stabilizing solution.
90 µL — Catalog Number C146-90UL

Assay Buffer
Assay buffer containing detergents and stabilizers.
50 mL — Catalog Number X117-50ML

Substrate
A solution of the substrate in a special stabilizing buffer.
5 mL — Catalog Number C129-5ML

Horseradish Peroxidase Concentrate
A 100X concentrated solution of HRP in a special stabilizing solution.
60 µL — Catalog Number X107-60UL

Galactose Oxidase
Freeze dried solution of Galactose Oxidase stored in a desiccator.
2 Vials — Catalog Number C147-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit. Once reconstituted, the Galactose Oxidase must be stored at -20°C.

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. See: www.arborassays.com/resources/#general-info for plate dimension data.

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°C or lower, preferably after being frozen in liquid nitrogen. Serum and plasma samples can be used after being diluted ≥ 1:15 in the supplied Assay Buffer. This assay has been validated for serum, plasma, buffer and media samples.

REAGENT PREPARATION

Horseradish Peroxidase (HRP) Preparation

Dilute the HRP Stock solution 1:100 with Assay Buffer using the table below:

	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

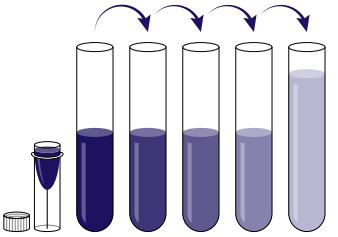
Allow the ziploc bag to warm completely to room temperature prior to opening. Remove the Galactose Oxidase vial and add 3.125 mL of the Assay Buffer to the vial. Vortex thoroughly. Each vial contains enough GOD for one plate.

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

REAGENT PREPARATION CONTINUED

Standard Preparation

Galactose Standards are prepared by labeling tubes as #1 through #6. Briefly vortex to mix the vial of Galactose Standard. 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 Standard 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.



	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

Use all Standards within 2 hours of preparation.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine sample concentrations.

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

1. Pipet 20 µL of diluted samples or standards into duplicate wells in the plate.
2. Pipet 20 µL of Assay Buffer into duplicate wells as the Zero standard.
3. Add 25 µL of the prepared HRP solution to each well using a repeater pipet.
4. Add 25 µL of the Substrate solution to each well using a repeater pipet. 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 standard and sample. Subract the average OD for the Zero from each duplicate to generate Net OD. Create a standard 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.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-galactose-colorimetric-detection-kit.assay

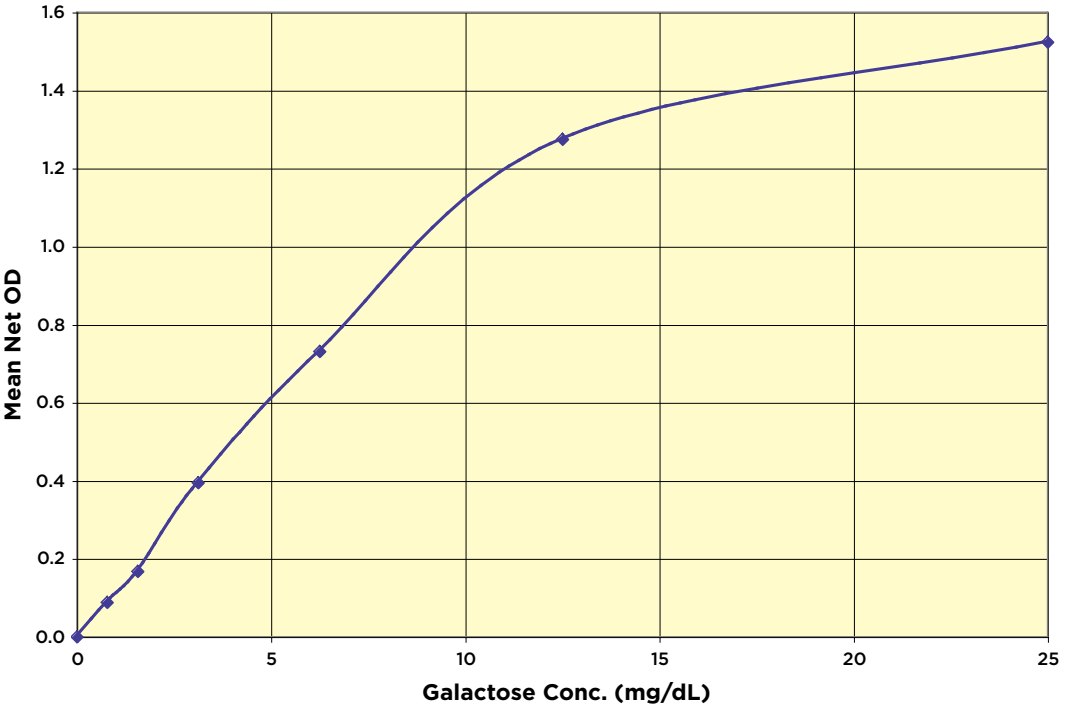
TYPICAL DATA

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 standard curves for calculation of results. Do not use this data.

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

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

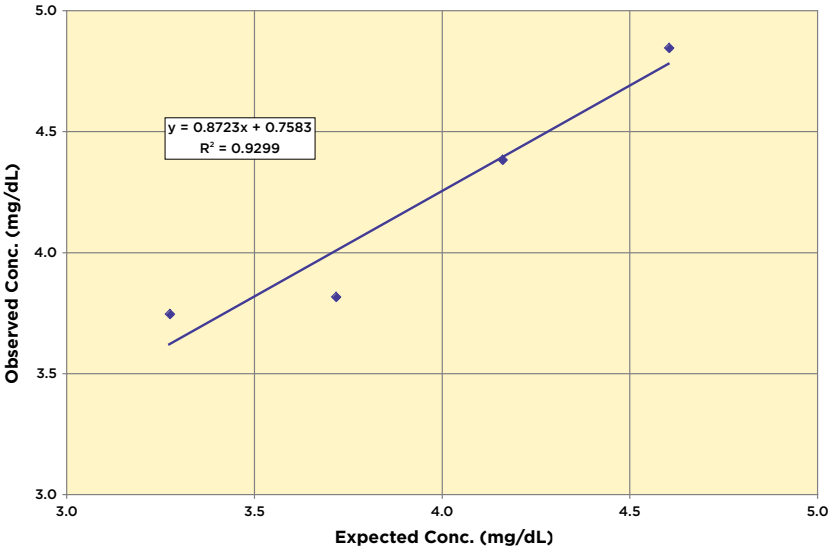
Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.493 mg/dL.**

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

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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