Galactose Colorimetric Detection Kit

Catalog Number EIAGALC (192 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Galactose Colorimetric Detection Kit is designed to measure galactose in serum, plasma, buffered solutions, and tissue culture medium. The kit uses galactose oxidase to react with galactose to produce hydrogen peroxide which, in the presence of horseradish peroxidase, reacts with a colorless substrate to produce a colored product. The assay was characterized with human samples, but can be used to test samples from other species.

Galactose is a hexose sugar that as an anomeric mixture of α-D-galactose and ß-D-galactose. Galactose is abundant in milk, dairy products, and many other food types such as fruits and vegetables, and is absorbed 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.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Galactose Standard; 250 mg/dL galactose in a special stabilizing solution	90 µL
Clear 96-well Half Area Plate	2 plates
Assay Buffer; contains detergent and stabilizers	50 mL
Substrate	5 mL
Horseradish Peroxidase Concentrate; 100X HRP in a special stabilizing solution	60 µL
Galactose Oxidase; freeze dried solution of galactose oxidase	2 vials

Materials required but not supplied

- Microtiter plate reader with software capable of measurement at or near 560 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute samples

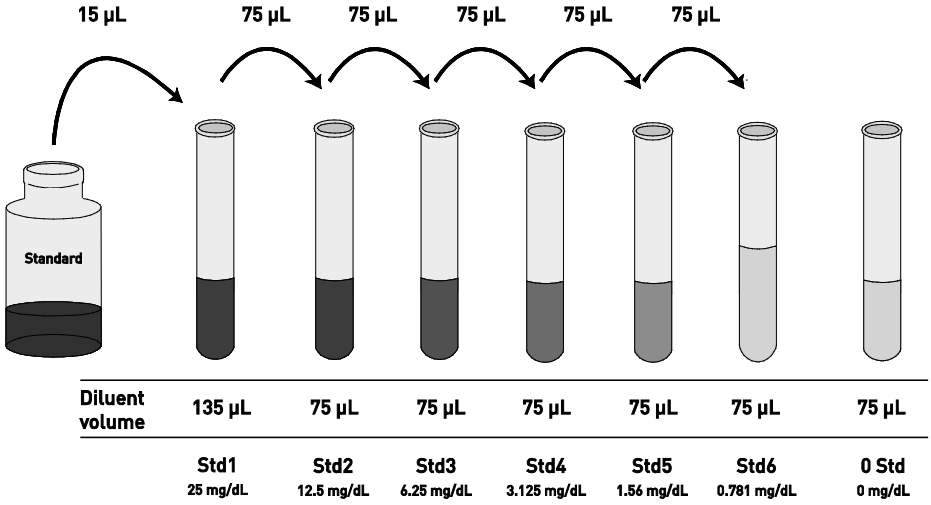
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute **serum** and **plasma** samples ≥1:15 in Assay Buffer.
- Perform sample dilutions with Assay Buffer.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly vortex the vial of standard to ensure the contents are mixed.
2. Add 15 µL Galactose Standard to one tube containing 135 µL Assay Buffer and label as 25 mg/dL galactose.
3. Add 75 µL Assay Buffer to each of 6 tubes labeled as follows: 12.5, 6.25, 3.125, 1.56, 0.781, and 0 mg/dL galactose.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.



Prepare 1X HRP solution

Dilute Horseradish Peroxidase Concentrate (100X) 1:100 with Assay Buffer.

Reagent	½ plate	1 plate	2 plates
Horseradish Peroxidase Concentrate (100X)	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

Reconstitute Galactose Oxidase

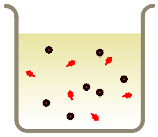
Note: Each vial contains enough galactose oxidase for one plate.

1. Allow the Galactose Oxidase to reach room temperature in the sealed bag before opening.
2. Add 3.125 mL of the Assay Buffer to the vial of Galactose Oxidase and vortex thoroughly.
3. Store any unused reconstituted Galactose Oxidase at –20°C.

Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

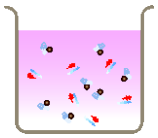
IMPORTANT! Perform a standard curve with each assay.



Add sample and HRP solution

a) Add 20 µL of standards or diluted samples (see page 2) to the appropriate wells.

b) Add 25 µL 1X HRP solution into each well.



Add substrate

a. Add 25 µL Substrate into each well.

b. Add 25 µL reconstituted Galactose Oxidase into each well

c. Incubate for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the absorbance at 560 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–25 mg/dL galactose.

Standard Galactose (mg/dL)	Optical Density (560 nm)
25	1.623
12.5	1.374
6.25	0.830
3.125	0.494
1.56	0.267
0.781	0.187
0	0.099

Note: 100 mg/dL of galactose is equivalent to 5.55 mM galactose.

Intra-assay precision

Three samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/dL)	9.58	5.75	3.18
%CV	4.8	3.6	6.2

CV = Coefficient of Variation

Inter-assay precision

Three samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/dL)	8.50	5.41	2.95
%CV	8.4	5.1	4.8

CV = Coefficient of Variation

Performance characteristics, continued

Linearity of dilution

Linearity was determined by assaying samples with high and low concentrations of galactose (high sample 5.05 mg/dL; low sample 2.83 mg/dL) mixed in the ratios shown in the following table.

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%**

Sensitivity

The analytical sensitivity of the assay is 0.493 mg/dL galactose. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

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