

Galactose & Lactose Assay Kit



Fast, convenient kit for sensitive colorimetric/fluorometric measurement of Galactose and Lactose in a variety of samples

Instruction Manual

Catalog Number	PK-CA577-K617																					
Description	<p>Galactose (C₆H₁₂O₆ FW: 180.16) and lactose (C₁₂H₂₂O₁₁ FW: 342.3) are naturally occurring sugars. Lactose consists of one galactose and one glucose. Some people, particularly infants, lack the enzyme necessary to digest galactose leading to galactose accumulation in blood (Galactosemia) causing enlarged liver, renal failure, cataracts and brain damage.</p> <p>PromoCell's <i>Galactose/Lactose Assay Kit</i> provides a simple, convenient tool for direct measurement of galactose and lactose levels in various biological samples (serum, plasma, other body fluids, food, growth media, etc.). To detect galactose, galactose oxidase specifically oxidizes free galactose, generating a product that reacts with the <i>Galactose Probe</i> to generate an absorbance change (OD = 570 nm) and fluorescence (Ex/Em 535/587). To detect lactose, lactose is hydrolyzed enzymatically by lactase to generate free galactose which is then measured. Thus, Lactose level = Total Galactose – Free Galactose.</p>																					
Applications	<ul style="list-style-type: none">Galactose and Lactose quantification in various biological samples																					
Sample Type	<ul style="list-style-type: none">Biological fluids (e.g. serum, plasma), growth media and food (e.g. milk)																					
Quantity	100 assays																					
Kit Components	<table border="1"><thead><tr><th>Components</th><th>Quantity</th><th>Color Code</th></tr></thead><tbody><tr><td>Galactose Assay Buffer</td><td>25 ml</td><td>WM</td></tr><tr><td>Galactose Probe (in DMSO)</td><td>200 µl</td><td>Red</td></tr><tr><td>Galactose Enzyme Mix (lyophilized)</td><td>1 vial</td><td>Green</td></tr><tr><td>Lactase (lyophilized)</td><td>1 vial</td><td>Blue</td></tr><tr><td>HRP (lyophilized)</td><td>1 vial</td><td>Purple</td></tr><tr><td>Choline Standard (5 µmol)</td><td>100 µl</td><td>Yellow</td></tr></tbody></table>	Components	Quantity	Color Code	Galactose Assay Buffer	25 ml	WM	Galactose Probe (in DMSO)	200 µl	Red	Galactose Enzyme Mix (lyophilized)	1 vial	Green	Lactase (lyophilized)	1 vial	Blue	HRP (lyophilized)	1 vial	Purple	Choline Standard (5 µmol)	100 µl	Yellow
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User Supplied Reagents & Equipment	<ul style="list-style-type: none">Microplate reader capable of absorbance and fluorescence measurement96-well clear (absorbance), black or white (fluorescence) plates with flat bottom																					
Storage and Reagents Preparation	<p>Store Kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Allow reagents warm to room temperature and read entire protocol before performing the assay.</p> <ul style="list-style-type: none">Galactose Probe: Ready to use as supplied. Warm to room temperature before use. Store at -20°C, protect from light and moisture. Use within two months.Lactase: Dissolve in 220 µl Galactose Assay Buffer. Aliquot and store at -20°C. Use within two months.Galactose Enzyme Mix: Dissolve in 220 µl Galactose Assay Buffer. Aliquot and store at -20°C. Use within two months.HRP: Dissolve in 220 µl Galactose Assay Buffer. Aliquot and store at -20°C. Use within two months.																					
Assay Protocol	<p>1. Standard Curve Preparation: <u>For the colorimetric assay</u>, dilute the Galactose Standard to 1 nmol/µl by adding 10 µl of the Galactose Standard to 990 µl of Galactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust the volume to 50 µl/well with Galactose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Galactose Standard.</p> <p><u>For the fluorometric assay</u>, dilute the Galactose Standard solution to 0.1 nmol/µl by adding 10 µl of the Galactose Standard to 990 µl of Galactose Assay Buffer and mix well. Then take 20 µl into 180 µl of Galactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Galactose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Galactose Standard.</p> <p>2. Sample Preparation: Liquid samples can be directly added into the plate, then adjusted to 50 µl each with Galactose Assay Buffer. Milk contains 4-9% lactose, 0.01 to 0.1 µl of milk can be measured. For unknown samples, we suggest testing several doses of sample to make sure the readings are within the standard curve linear range. If you want to detect Lactose, prepare two wells for each sample. To one well add 2 µl of Lactase to convert lactose to free galactose for detecting total galactose, incubate at 37°C for 30 minutes. Add no lactase to the other well for</p>																					

detecting free galactose. Then, **Lactose = Total Galactose – Free Galactose.**

3. Galactose Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

- 44 µl Galactose Assay Buffer
- 2 µl Galactose Probe
- 2 µl Galactose Enzyme Mix
- 2 µl HRP

Mix well. Add 50 µl of the Reaction Mix to each well containing the Galactose Standard and test samples. Mix well. Incubate the reaction for 60 minutes at 37°C, protected from light.

4. Measurement: Measure at OD570 nm for the colorimetric assay or at Ex/Em = 535/590 nm for the fluorometric assay in a microplate reader.

5. Calculation: Correct background by subtracting the value of the 0 galactose control from all readings. Plot standard curve. Apply sample readings to the standard curve. Calculate galactose concentration:

$$C = Ga/Vs \text{ [nmol/}\mu\text{l or mM]}$$

Where Ga: Galactose amount in the sample wells from standard curve (nmol).

Vs: Volume of sample added into the wells (µl).

Intended Use

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

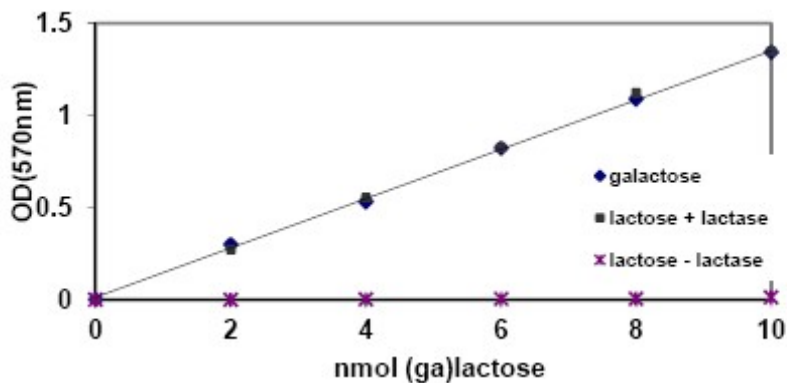


Figure: Colorimetric measurement of Galactose and Lactose using the PromoCell assay.

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