Galactose and Lactose Colorimetric/Fluorometric Assay Kit

(Catalog #K617-100; 100 assays; Store at -20°C)

I. Introduction:

Galactose ($C_6H_{12}O_6$ FW: 180.16) and lactose ($C_{12}H_{22}O_{11}$ 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. BioVision's Galactose / Lactose Assay Kit provides a simple, convenient means 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 Galactose Probe to produce color (OD 570nm) and fluorescence (Ex/Em 535/587). To detect lactose, lactose is hydrolyzed by lactase to generate free galactose which is then measured. Thus, Lactose level = Total Galactose – Free Galactose.

II. Kit Contents

Components	K617-100	Cap Code	Part No.
Galactose Assay Buffer	25 ml	WM	K617-100-1
Galactose Probe (DMSO solution)	0.2 ml	Red	K617-100-2A
Lactase (Lyophilized)	1 Vial	Blue	K617-100-4
Galactose Enzyme Mix (Lyophilized)	1 Vial	Green	K617-100-5
HRP (Lyophilized)	1 Vial	Purple	K617-100-6
Galactose Standard (100nmol/µl)	100 µl	Yellow	K617-100-7

III. Storage and Handling:

Store kit at –20°C, protect from light. Allow reagents warm to room temperature and briefly centrifuge vials before opening.

IV. Reagent Preparation:

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.

V. Galactose Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, 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.

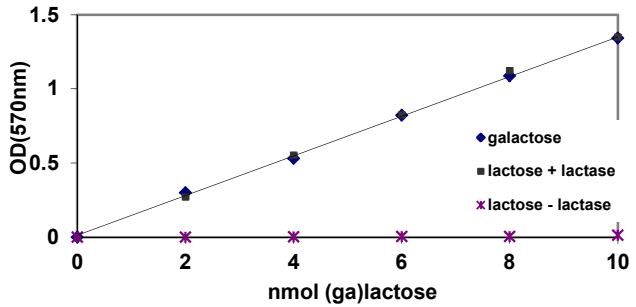
For the fluorometric assay, dilute the Galactose Standard solution to 0.1 nmol/µl by adding 10 µl of the Galactose Standard to 990 µl of Galactose Assay Buffer and mix well. Then take 20 µl into 180 µl of Galactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust 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.

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 min. Add no lactase to the other well for 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
4. Mix well. Add 50 µl of the Reaction Mix to each well containing the Galactose Standard and test samples. Mix well.
5. Incubate the reaction for 60 minutes at 37°C, protect from light.
6. Measure OD 570nm for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microplate reader.
7. **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 nmol/µl or mM

   Where Ga: Galactose amount in the sample wells from standard curve (nmol).
   Vs: Volume of sample added into the wells (µl).



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NADH/NADPH Assay Kit
Uric Acid assay kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		