Product Information

Galactose Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2126-100 100 assays	Cap Color	Part Number
Galactose Assay Buffer	25 ml	WM	K2126-C-1
Galactose Probe (DMSO solution)	0.2 ml	Red	K2126-C-2
Galactose Enzyme Mix (Lyophilized)	1 Vial	Green	K2126-C-3
HRP (Lyophilized)	1 Vial	Purple	K2126-C-4
Galactose Standard (100 nmol/µl)	100 µl	Yellow	K2126-C-5

II. Introduction:

Galactose ($C_6H_{12}O_6$, Gal) is a monosaccharide sugar and is a naturally occurring sugar that involved in many biological processes. Galactose combines with glucose to form lactose that is mainly present in milk and milk products.

The Galactose Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of galactose levels in various biological samples (plasma, serum, growth media, other body fluids, food, etc.) based on colorimetric and fluorometric method. In the assay, galactose is specifically oxidized producing a product that yields fluorescence (Ex/Em 535/587 nm) and color (λmax = 570 nm). Liquid samples can be tested directly without purification. The kit is also suited for a high throughput assay.

III. Storage and Handling:

Store kit at -20°C, protect from light. Briefly centrifuge vials prior to opening. Allow buffer warm to room temperature before use, but keep enzymes on ice during the assay.

IV. Reagent Preparation:

Galactose Probe: Ready to use as supplied. Warm to room temperature prior to use. Store at -20°C, protect from light and moisture. 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 100 nmol/µl 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 adjust to a total volume of 50 µl with Galactose Assay Buffer. For unknown samples, we suggest testing several doses of sample to ensure the readings are within the standard curve linear range.

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

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

Note: The fluorometric assay is ~10 fold more sensitive than the colorimetric assay. Use 0.4 µl of the probe in each reaction in the fluorometric assay to decrease background fluorescence significantly.

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 30 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.

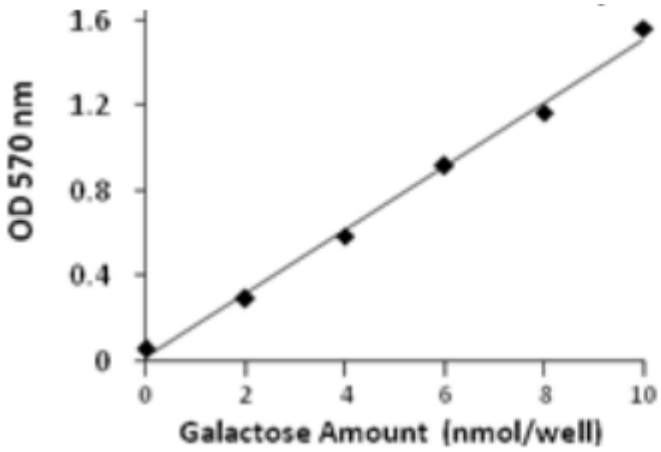
7. Calculations: Correct background by subtracting the value of the 0 galactose control from all sample readings. Plot standard curve galactose amount (nmol) vs OD 570nm. Apply sample readings to the standard curve. Galactose concentration:

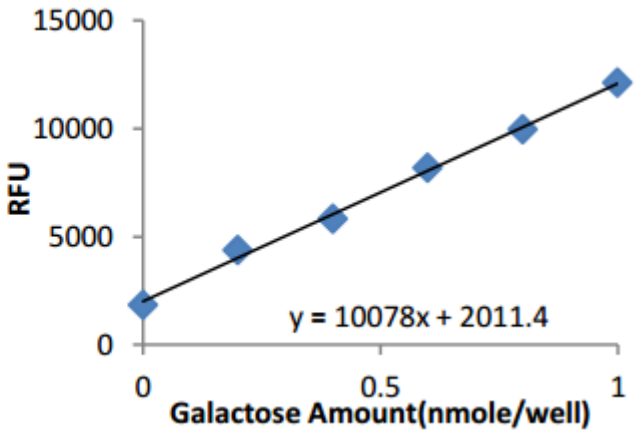
C = Ga/Sv nmol/µl or mM

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

Sv: Sample volume added into the sample wells (in µl).

Galactose Molecular Weight: 180.16.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions

	atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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