ab83382

Galactose Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Galactose levels in various samples.

This product is for research use only and is not intended for diagnostic use.

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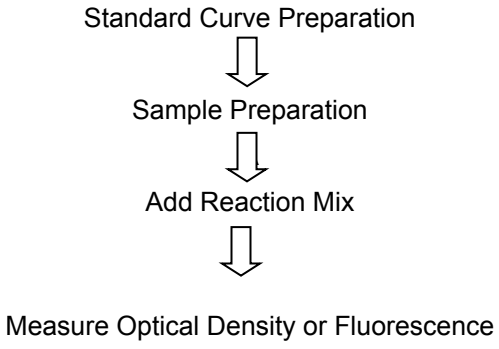
1. Overview

Galactose ($C_6H_{12}O_6$ FW: 180.16) is one the major naturally occurring sugars, involved in many biological processes. Abcam's Galactose Assay Kit provides a simple, convenient means for direct measurement of galactose levels in various biological samples (serum, plasma, other body fluids, food, growth media, etc.).

In the assay kit, Galactose is oxidized generating a product that produces color ($OD_{570\ nm}$) and fluorescence (Ex/Em 535/587 nm). Liquid samples can be tested directly without purification. The assay is fast, convenient and sensitive. It can also be used as a high throughput assay.

High concentrations of lactose could result in elevated readings for free galactose. Galactose oxidase is not completely selective, and it can react with the galactose moiety of lactose. The enzyme reactivity towards lactose is considerably lower than towards galactose, while highly dependent on the temperature and reaction/incubation time. After 40 min incubation at 22°C, lactose will exhibit 15% reactivity, when compared to equimolar amounts of galactose.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 2	25 mL
OxiRed™ Probe	0.2 mL
Galactose Enzyme Mix	1 vial
Galactose Standard	100 µL
Developer Solution V	1 vial

PLEASE NOTE: Developer Solution V was previously labelled as HRP (Lyophilized), and Assay Buffer 2 as Assay Buffer II and Galactose Assay Buffer, and OxiRed™ Probe as Galactose Probe (DMSO solution). The composition has not changed.

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

OxiRed™ Probe: Ready to use as supplied. Allow to 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 Assay Buffer 2. Aliquot and store at -20°C. Use within two months.

Developer Solution V: Dissolve in 220 µL Assay Buffer 2. Aliquot and store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well black plate (fluorometric assay), 96 well clear plate (colorimetric assay)
- Orbital shaker

4. Assay Protocol

1. Standard Curve Preparation:

a. 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 Assay Buffer 2 and mix well.

Add 0, 2, 4, 6, 8, 10 µL into a series of wells. Adjust the volume to 50 µL/well with Assay Buffer 2 to generate 0, 2, 4, 6, 8, 10 nmol/well of Galactose Standard.

b. 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 Assay Buffer 2 and mix well. Then take 20µL into 180 µL of Assay Buffer 2 and mix well.

Add 0, 2, 4, 6, 8, 10 µL into each well individually. Adjust volume to 50 µL/well with Assay Buffer 2 to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Galactose Standard.

2. Sample Preparation:

a. Liquid samples: can be directly added into the plate, then adjust to a total volume of 50 µL with Assay Buffer 2.

b. Tissue or cell samples: 20-50 mg of tissue or 1 million cells should be rapidly homogenized with 100 µL of lysis buffer (pH 6.5 – 8). Centrifuge at 13000 rpm for 10 min to remove insoluble materials.

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 samples and standards to be performed. For each well, prepare a total 50 µL Reaction Mix:

Assay Buffer 2	44 µL
OxiRed™ Probe*	2 µL
Galactose Enzyme Mix	2 µL
Developer Solution V	2 µL

*** Note:** The fluorometric assay is ~10 fold more sensitive than the colorimetric assay. Use 0.4 µL of the OxiRed™ 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 40 minutes at 22°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.

Notes: Longer incubation times or higher temperature will result in increased reactivity towards lactose.

5. Data Analysis

Correct background by subtracting the value of the zero galactose control from all sample and standard readings.

Plot standard curve galactose: amount (nmol) vs OD_{570nm} (Ex/Em = 535/590 nm). Apply sample readings to the standard curve.

Galactose concentration:

Concentration = Ga / Sv (nmol/µl or mM)

Where:

Ga is Galactose amount from standard curve (nmol).

Sv is the sample volume added in sample wells (µl).

Galactose Molecular Weight: 180.16.

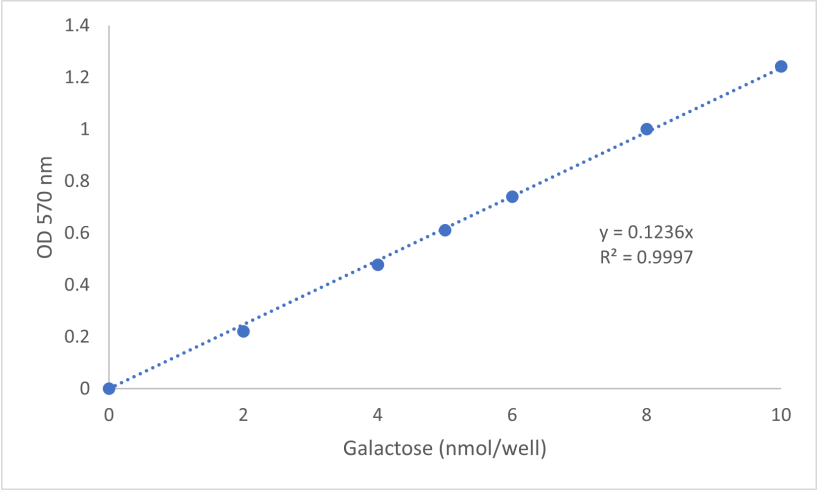


Figure 1. Galactose Standard Curve – Colorimetric mode.

Example of galactose standard curve obtained with ab83382 in colorimetric mode.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes

	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Notes:

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)