

## Galactose Assay Kit

(Catalog #K621-100; 100 assays; Store at -20°C)

46  $\mu$ l Galactose Assay Buffer  
2  $\mu$ l Galactose Probe\*  
2  $\mu$ l Galactose Enzyme Mix

### I. Introduction:

Galactose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> FW: 180.16) is one the major naturally occurring sugars, involved in many biological processes. BioVision'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 was specifically oxidized generating a product that reacts with the Galactose Probe to produce color (O.D 570 nm) and fluorescence (Ex/Em 535/587 nm). Therefore, galactose can be easily quantified. Liquid samples can be tested directly without purification. The assay is fast, convenient and sensitive. It can also be used in high throughput assay.

### II. Kit Contents

Components	K621-100	Cap Code	Part No.
Galactose Assay Buffer	25 ml	WM	K621-100-1
Galactose Probe (Lyophilized)	1 Vial	Red	K621-100-2
Dimethylsulfoxide (DMSO, Anhydrous)	0.4 ml	Brown	K621-100-3
Galactose Enzyme Mix (Lyophilized)	1 Vial	Green	K621-100-4
Galactose Standard (100 nmol/ $\mu$ l)	100 $\mu$ l	Yellow	K621-100-5

### 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:** Dissolve in 220  $\mu$ l DMSO (provided) before use. Store at -20°C, protect from light and moisture. Use within two months.

**Galactose Enzyme Mix:** Dissolve in 220  $\mu$ l Galactose Assay Buffer. Aliquot and store at -20°C. Use within two months.

### V. Galactose Assay Protocol:

#### 1. Standard Curve Preparation:

For colorimetric assay, dilute the Galactose Standard to 1 nmol/ $\mu$ l by adding 10  $\mu$ l of the 100 nmol/ $\mu$ l Galactose Standard to 990  $\mu$ l of Galactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10  $\mu$ l into a series of wells. Adjust the volume to 50  $\mu$ l/well with Galactose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Galactose Standard.

For fluorometric assay, dilute the Galactose Standard solution to 0.1 nmol/ $\mu$ l by adding 10  $\mu$ l of the Galactose Standard to 990  $\mu$ l of Galactose Assay Buffer and mix well. Then take 20  $\mu$ l into 180  $\mu$ l of Galactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10  $\mu$ l into each well individually. Adjust volume to 50  $\mu$ l/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:** Different amounts of sample can be directly added into the plate, then adjust to total volume to 50  $\mu$ l 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.

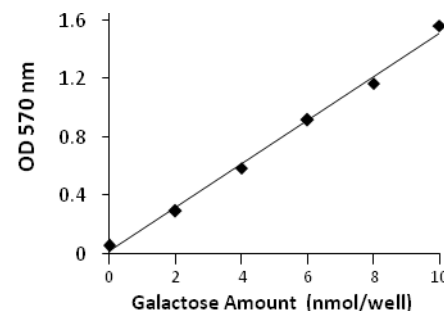
3. **Galactose Reaction Mix:** Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50  $\mu$ l Reaction Mix containing:

\***Note:** The fluorometric assay is ~10 fold more sensitive than the colorimetric assay. Use 0.4  $\mu$ l probe in each reaction in the fluorometric assay will decrease background fluorescence reading significantly.

- Mix well. Add 50  $\mu$ l of the Reaction Mix to each well containing the Galactose Standard and test samples. Mix well.
- Incubate the reaction for 30 minutes at 37°C, protect from light.
- Measure O.D. 570nm for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microplate reader.
- 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:

$$C = \text{Ga/Sv} \text{ nmol}/\mu\text{l or mM}$$

Where Ga: Galactose amount in the sample wells (in nmol).  
Sv: Sample volume added into the sample wells (in  $\mu$ l).  
Galactose Molecular Weight: 180.16.



### VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents  
Cell Proliferation & Senescence  
Metabolism Assay Kits  
Maltose Assay Kit  
Fatty Acid Assay Kit  
Ethanol Assay Kit  
NAD(P)/NAD(P)H Assay Kit  
Cretine & Cretinine Assay Kits  
Sarcosine Assay Kit  
Nitric Oxide Assay Kits

Cell Fractionation Kits  
Cholesterol and Obesity Assay Kits  
Glucose/Sucrose Assay Kit  
L-Amino Acid assay Kit  
NADH/NADPH Assay Kit  
ADP/ATP Assay Kit  
Uric Acid assay kit  
Calcium Assay Kit  
Glutathione Assay Kit  
SOD Assay Kit