

# Fructose Assay Kit

(Catalog # K619-100; 100 assays; Store kit at -20°C)

## I. Introduction:

Fructose is a monosaccharide found in many foods and is one of the three most important blood sugars along with glucose and galactose. Fructose is the sweetest naturally occurring sugar, estimated to be twice as sweet as sucrose. In BioVision's Fructose Assay Kit, free fructose is enzymatically converted to  $\beta$ -glucose, which is then specifically oxidized to generate a product that reacts with OxiRed Probe to generate color ( $\lambda=570\text{nm}$ ) and fluorescence (Ex/Em=535/587nm). The kit provides a rapid, simple, sensitive, and reliable method suitable for high throughput assay of D-fructose.

## II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Fructose Assay Buffer	25 ml	WM	K619-100-1
OxiRed Probe	1 vial	Red	K619-100-2
Dimethylsulfoxide (DMSO; Dried)	0.4 ml	Brown	K619-100-3
Enzyme Mix (lyophilized)	1 vial	Green	K619-100-4
Fructose Converting Enzyme	1 vial	Purple	K619-100-5
Fructose Standard (100 mM)	100 $\mu\text{l}$	Yellow	K619-100-6

## III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

## IV. Reagent preparation:

**Probe:** Dissolve in 220  $\mu\text{l}$  DMSO (provided) before use. Store at -20°C, protect from light and moisture.

**Enzyme Mix, Fructose Converting Enzyme:** Dissolve in 220  $\mu\text{l}$  Assay Buffer separately. Store at -20°C. Use within two months.

## V. Fructose Assay Protocol:

### 1. Standard Curve Preparation:

**For the colorimetric assay,** dilute the 100 mM Fructose Standard solution to 1 mM by adding 10  $\mu\text{l}$  of Fructose Standard to 990  $\mu\text{l}$  of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into each well individually. Adjust volume to 50  $\mu\text{l}$ /well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Fructose Standard.

**For the fluorometric assay,** dilute the Fructose Standard solution to 0.1 mM by adding 10  $\mu\text{l}$  of the Fructose Standard to 990  $\mu\text{l}$  of Assay Buffer, mix well. Then take 10  $\mu\text{l}$  of the diluted Fructose Standard into 90  $\mu\text{l}$  of Fructose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into each well individually. Adjust volume to 50  $\mu\text{l}$ /well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1 nmol/well of the Fructose Standard.

- 2. Sample Preparations:** Tissues or cells can be homogenized in the Assay Buffer centrifuge to remove insoluble material at 13,000 rpm, 10 minutes. Serum sample can be directly diluted in the Assay Buffer. Prepare test samples in 50  $\mu\text{l}$ /well with Assay Buffer in a 96-well plate. For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

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

44  $\mu\text{l}$  Assay Buffer  
 2  $\mu\text{l}$  OxiRed Probe\*\*  
 2  $\mu\text{l}$  Enzyme Mix  
 2  $\mu\text{l}$  Fructose Converting Enzyme\*

Mix well. Add 50  $\mu\text{l}$  of the **Reaction Mix** to each well containing the Fructose Standard and test samples. Mix well. Incubate the reaction for 1 hour at 37°C, protect from light.

**Note:** \*Glucose generates background. If glucose is in your sample, the glucose background can be subtracted by doing a control without Fructose Converting Enzyme in the reaction. The glucose background reading can be subtracted from the sample reading that contains Converting enzyme to get fructose reading.

\*\*The fluorometric assay is 10 fold more sensitive. In the fluorometric assay, 0.4  $\mu\text{l}$  of the OxiRed probe can be used for each reaction to reduce the background fluorescence readings.

- 4.** Measure O.D. 570 nm for colorimetric assay or Ex/Em = 535/587 nm for fluorometric assay in a micro plate reader.
- 5.** Correct background by subtracting the value derived from the 0 fructose control from all sample readings (The background reading can be significant and must be subtracted from sample readings). Plot fructose standard Curve, fructose concentrations of the test samples can then be calculated:

$$C = S_a/S_v \text{ nmol}/\mu\text{l} \text{ or mM,}$$

where  $S_a$  is the sample amount of unknown (in nmol) from standard curve,

$S_v$  is sample volume ( $\mu\text{l}$ ) added to the wells.

Fructose Molecular Weight is 180.16 g/mol.

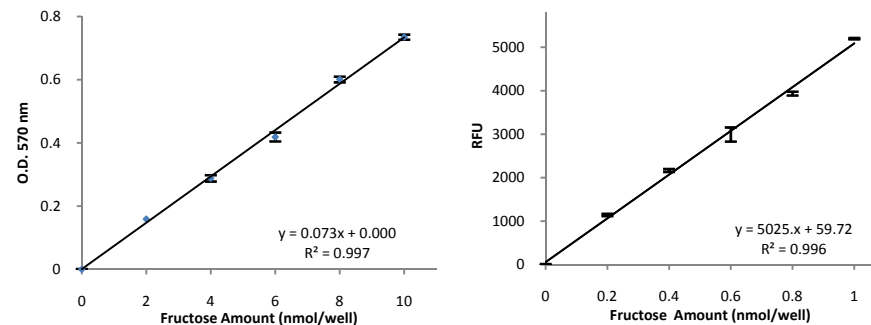


Figure: Fructose Standard Curve. Assays were performed follow the kit protocol.

## VI. Related Products:

Glucose Assay Kit  
 Galactose Assay Kit  
 Pyruvate Assay Kit  
 Fatty Acid Assay Kit  
 Cholesterol Assay Kit

Sucrose Assay Kit  
 Glycerol Assay Kit  
 Lactate Assay Kit  
 Triglyceride Assay Kit  
 Glutathione Assay Kit