

Fructose Assay Kit

(Cat/No.:BC166 Size:50T/48S)

1. Principle of Measurement

Fructose reacts with the substrate solution (reagent I) and the product has a maximum absorbance value at 285 nm. The absorbance can be measured by UV-spectrophotometer and thus the amount of fructose can be measured.

2. Compositions and Preparation (The kit is valid for 6 months)

Reagent I: Substrate. 2 Bottles×75 ml. Preserved at 4 °C.

Reagent II: Fructose Standard. 3 Bottles of D-Fructose Powder.

Preparation of Reagent II Solution: Dissolve each bottle of powder with distilled water to 10 ml final volume. The final concentration is 1mg/ml.

3. Procedures

Compositions (ml)	Blank	Standard	Sample
Double Distilled Water (DDW)	0.05		
Reagent II Solution		0.05	
Sample Solution			0.05
Reagent I	3	3	3

Mix thoroughly and boil the mixture in a water bath for 8 min. Cool the mixture with water and zero the cuvettes at 285 nm with 1 cm path length using DDW. Record the optical density (OD) value of each tube at 285 nm.

4. Pre-treatment and Calculations

1. For Juice Samples

a. Pretreatment

Take the freshly prepared juice and centrifuge at 3,500 rpm for 10 min. Extract the supernatant for further measurement.

b. Formula

$$C_{Fructose} \frac{mg/ml}{mg/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD$$

Note: CoD represents the coefficient of dilution in the pretreatment process.

c. Example

Freshly prepared cantaloupe juice was centrifuged and the supernatant was diluted with DDW to 100 times of its initial volume. The OD values were 0.014, 0.371 and 0.129 respectively.

$$C_{Fructose} \frac{mg/ml}{mg/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD = \frac{0.129 - 0.014}{0.371 - 0.014} \times 1 \times 100 = 32.21mg/ml$$

**2. Honey Samples**

a. Pretreatment

Samples should be diluted with DDW to 1000 times of its initial volume for measurement.

b. Formula

$$C_{Fructose} \frac{mg}{ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD$$

c. Example

Honey sample was diluted with DDW to 1000 times of its initial volume and measured. The OD values were 0.014, 0.371 and 0.372 respectively.

$$C_{Fructose} \frac{mg}{ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD = \frac{0.372 - 0.014}{0.371 - 0.014} \times 1 \times 1000 = 1.003 \times 10^3 mg/ml$$

3. Semen Samples

a. Pretreatment

Pretreat the sample through liquefaction process and centrifuge the mixture at 2,500 rpm for 15 min. Extract the supernatant for measurement.

b. Formula

$$C_{Fructose} \frac{mg}{ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD$$

c. Example

Dilute the pre-treated semen sample with DDW to 5 times of its initial volume. Then the sample was measured with OD values 0.014, 0.371 and 0.342 respectively.

$$C_{Fructose} \frac{mg}{ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \times CoD = \frac{0.342 - 0.014}{0.371 - 0.014} \times 1 \times 5 = 4.590 mg/ml$$

4. Tissue Samples

a. Pretreatment

Weigh the tissue precisely and add physiological saline with the ratio 1 g tissue to 4 ml saline. Homogenize the mixture and centrifuge the homogenate at 2,500 rpm for 10 min. Note that turbidity may appear for the high concentration and under such circumstance, centrifugation is recommended to be done with 4,000 rpm with the period of 10 min.

b. Formula

$$C_{Fructose} \frac{mg}{gprot} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \div \frac{C_{Protein}}{g/ml}$$

c. Example

Rat testicle tissue was pre-treated and measured. The OD values were 0.014, 0.371 and 0.139 respectively. The protein concentration of the homogenate was measured to be 9.340mg/ml.

$$\frac{C_{Fructose}}{mg/gprot} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1mg/ml} \div \frac{C_{Protein}}{g/ml} = \frac{0.139 - 0.014}{0.371 - 0.014} \times 1 \div (9.340 \div 1000) = 37.49 \text{ mg/g}$$

5. Significance of Measurement

Fructose in vivo is secreted by seminal vesicles and is the main saccharide components within semen as a major source of energy for sperm. Thus the fructose concentration significantly relates to the motility of sperm. The concentration will directly influence the motility of sperm and is a factor to judge the motility.

Fructose is the sweetest of all naturally occurring carbohydrates and is 1.73 times as sweet as sucrose. The fructose can be found in juice or honey and is a main factor to judge such products' quality. The method establishment of fructose measurement signify the quality control of juice and honey.

Appendix I: Standard Curve Establishment

1. Pre-Treatment

One bottle of fructose powder was dissolved with DDW to the final volume of 1 ml and the concentration was (10mg/ml). Then dilute the solution to solutions with concentrations of 0.0625, 0.125, 0.25, 0.5, 1, 2, 3, 4, 5 mg/ml.

2. Procedures

Compositions (ml)	Blank	Sample
DDW	0.05	
Fructose Solution		0.05
Reagent I	3	3

Mix thoroughly and boil the mixture for 8 min. Cool the solution and measure the OD value of each tube at 285 nm after zeroing the cuvettes with DDW.

3. Results

Concentration (mg/ml)	OD _{Sample}	Absolute OD (OD _{Sample} -OD _{Blank})
0	0.014	0
0.0625	0.035	0.021
0.125	0.061	0.047
0.25	0.106	0.092
0.5	0.195	0.181
1	0.371	0.357
2	0.682	0.668
3	0.993	0.979
4	1.315	1.301



5	1.623	1.609
---	-------	-------

4. Standard Curve

