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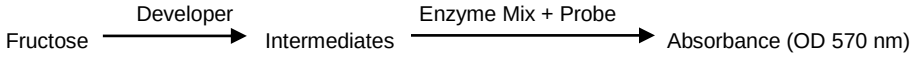
# Fructose Assay Kit (Colorimetric)



**(Catalog # K439-100; 100 assays; Store 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, although it has a much lower glycemic index than both sucrose and glucose. For these reasons, fructose has been investigated as a food sweetener, and its downstream metabolic effects are of considerable interest. Studies have shown that fructose levels may correlate with cardiometabolic disease, gout, and liver function. BioVision's Fructose Assay Kit utilizes an enzymatic mechanism by which a highly specific enzyme metabolizes fructose, and then a developer is used to generate the proportional colorimetric signal that can be quantified at 570 nm. The assay shows greater than 100-fold specificity for Fructose over glucose and other sugars, both mono- and polysaccharides. The method is suitable for use in a range of biological and culinary samples, and can detect as lower than 0.1 nmole (~15 ng) Fructose.




## II. Applications:

- Determination of Fructose concentration in biological samples e.g. tissue lysates
- Determination of Fructose levels in agricultural and culinary products

## III. Sample Type:

- Cell and Tissue Lysates
- Food and Beverage Samples

## IV. Kit Contents:

| Components | K439-100 | Cap Code | Part Number |
|---|---|---|---|
| Fructose Assay Buffer | 25 ml | NM | K439-100-1 |
| Fructose Developer Buffer | 5 ml | NM | K439-100-2 |
| Fructose Enzyme Mix | 1 vial | Blue | K439-100-3 |
| Fructose Substrate | 1 vial | Purple | K439-100-4 |
| Fructose Developer | 1 vial | Green | K439-100-5 |
| OxiRed™ Probe (in DMSO) | 200 µl | Red | K439-100-6 |
| Fructose Standard (100 mM) | 100 µl | Yellow | K439-100-7 |

## V. User Supplied Reagents & Equipment:

- Plate Reader capable of 37°C setting and absorbance readings
- 96-well clear plate with flat bottom

## VI. Storage and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Warm buffers to room temperature before use. Read entire protocol before performing the assay.

- **Fructose Assay Buffer and Developer Buffer:** Store at -20°C. Warm to RT before use. Stable for six months.
- **Fructose Enzyme Mix and Fructose Substrate:** Add 220 µl of Assay Buffer to each vial. Mix well. Store at -20°C. Use within six months.
- **Fructose Developer:** Add 220 µl of $dH_2O$ to Developer. Mix well. Store at -20°C. Use within six months.
- **Fructose Standard:** Ready to use as supplied. Warm to room temperature before use. Use within six months.
- **OxiRed Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Protect from light and use within six months.

## VII. Fructose Determination Assay Protocol:

1. **Sample Preparation:** Solid foods, tissues and cells can be homogenized in the Assay Buffer; use 100 µl **Assay Buffer** for every 10 mg tissue/1x$10^6$ cells. Using a dounce (BV # 1998) or other homogenizer, rapidly homogenize sample, with buffer, on ice. Clarify samples by centrifugation at 10,000 x g for 5 minutes at 4°C and use supernatant. Beverage samples may be assayed directly; dilution may be necessary (juices and other sweetened beverages may need to be diluted 100-fold or more). Pipet equal volume (2-20) µl of each sample into two wells: the Sample well and the Sample Background well (needed to determine background for each volume of sample) onto a 96-well clear plate. Bring the volume of each well to 30 µl with Fructose Assay Buffer.

   **Note:**

   **a.** Fructose concentration varies over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   **b.** While this assay can measure levels of fructose above 0.1 nmole reliably, many biological samples, such as serum and plasma, will have fructose levels on the cusp of this sensitivity. For these samples, we recommend using our Fructose PicoProbe™ Assay (K611).

2. **Standard Curve Preparation:** Prepare 1 mM Fructose Standard by adding 10 µl 100 mM Fructose Solution to 990 µl Fructose Assay Buffer. Mix thoroughly.

Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM Fructose Standard to each well individually to generate standards of 0, 2, 4, 6, 8, and 10 nmol Fructose/well. Adjust the volume of each well to 30 µl with Assay Buffer.

**Note:** We recommend generating a standard curve each time experiments are run, as small variations in components and timing can affect signal reading.

3. **Reaction Mix**: Mix enough reagent for the number of samples and standards to be performed: For each well (standards and one for each sample), prepare 30 µl Reaction Mix. For sample background wells, prepare 30 µl Background Control Mix:

| | Reaction Mix (per well) | Background Control Mix (per well) |
|---|---|---|
| Fructose Assay Buffer | 26 µl | 28 µl |
| Fructose Enzyme Mix | 2 µl | ----- |
| Fructose Substrate | 2 µl | 2 µl |

Add 30 µl Reaction Mix and 30 µl Background Control Mix to respective sample wells. Incubate plate at 37°C for 30 minutes.

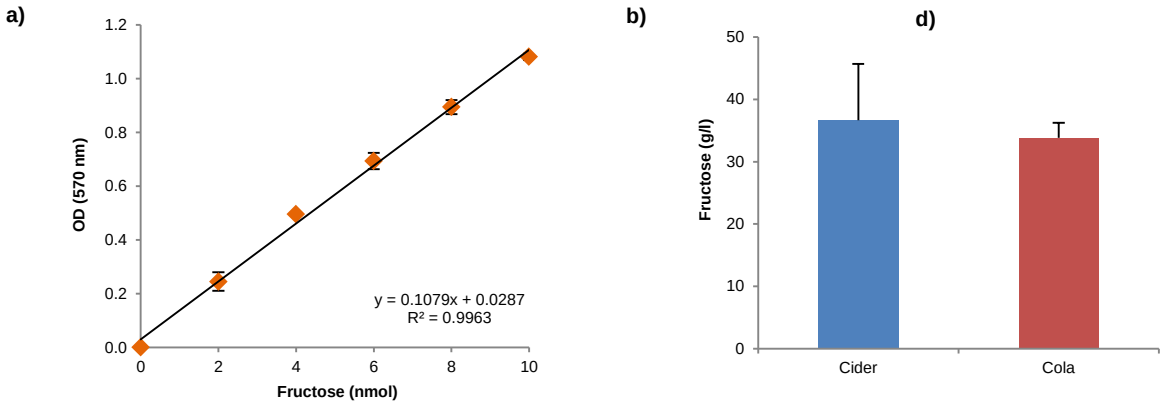
4. **Measurement:** Prepare enough Development Mix for all wells:

| | Development Mix (per well) |
|---|---|
| Fructose Developer Buffer | 36 µl |
| Fructose Developer | 2 µl |
| OxiRed Probe (in DMSO) | 2 µl |

Mix well and add 40 µl to each well. Incubate plate at 37°C for 5 minutes and read absorbance at 570 nm.

5. **Calculations:** Subtract the 0 Fructose Standard reading from all standard readings, and plot the background-subtracted Fructose Standards to generate the standard curve (from 0-10 nmol Fructose). For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Apply the background-subtracted values to the standard curve to calculate Fructose concentration:

$$\textbf{Fructose Concentration} = \left(\frac{\text{Fructose amount from standard curve (nmol)}}{\text{vol. of sample(ml)}}\right) x\ Dilution\ Factor\ D\ \left(\frac{nmol}{\mu l}\ or\ mM\right)$$




**Figures: (a) Fructose Standard Curve:** Following the Assay Protocol, slope was determined to be 0.1079 AU/nmole and $R^2$ was 0.996. **(b) Fructose in beverages:** Apple Cider or Cola from a soda fountain was diluted 1:200 with $dH_2O$, and 2-10 µl of the dilutions were assayed using the kit protocol. Values were averaged to give presented data.

## VIII. RELATED PRODUCTS:

Glucose Colorimetric/Fluorometric Assay Kit (K653)
Galactose Colorimetric/Fluorometric Assay Kit (K805)
Sucrose Colorimetric/Fluorometric Assay Kit (K626)
Lactate Colorimetric/Fluorometric Assay Kit (K607)
Free Glycerol Colorimetric/Fluorometric Assay Kit (K630)
PicoProbe Fructose Fluorometric Assay Kit (K611)
PicoProbe Fructose-6-Phosphate Fluorometric Assay Kit (K689)
PicoProbe Glucose-6-Phosphate Fluorometric Assay Kit (K751)
Maltose Colorimetric/Fluorometric Assay Kit (K628)
Glycerol Cell-Based Assay Kit (K977)

***FOR RESEARCH USE ONLY! Not to be used on humans***