

## **Technical Manual**

## **ChromaDazzle Fructose Assay Kit**

**Catalogue Code: BA0109**

**Pack Size: 100 assays**

**Research Use Only**

## DESCRIPTION

**FRUCTOSE** (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, also called levulose or laevulose), is a monosaccharide found in honey, tree fruits, berries, melons, and some root vegetables along with glucose and galactose. The human body can use fructose for energy, however, too much consumption may lead to high triglycerides. Simple, direct and high-throughput assays for fructose determination find wide applications. The Assay Genie ChromaDazzle Fructose Assay Kit reagent reacts directly and specifically with fructose to form a colored product. Glucose and galactose do not interfere. The color intensity at 565nm is directly proportional to the fructose concentration in the sample.

## KEY FEATURES

Use as little as 20  $\mu$ L samples. Linear detection range in 96-well plate: 12 to 1000  $\mu$ M fructose.

## APPLICATIONS

**Direct Assays:** fructose in biological samples (e.g. serum, plasma, urine, saliva, milk, culture medium), food, juice, beverage and other agricultural products.

**Drug Discovery/Pharmacology:** effects of drugs on fructose metabolism.

## KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

<b>Assay Buffer:</b>	10 mL	<b>Enzyme:</b>	Dried
<b>PMS Solution:</b>	1.5 mL	<b>Enzyme Buffer:</b>	150 $\mu$ L
<b>MTT Solution:</b>	1.5 mL	<b>Standard:</b>	400 $\mu$ L 20 mM D-Fructose

**Storage conditions.** The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## ASSAY PROCEDURE

*Note: (1) The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%). (2) This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.*

**Reagent Preparation:** Reconstitute Enzyme by adding 120  $\mu$ L Enzyme Buffer to the Enzyme tube. Make sure Enzyme is fully dissolved by pipetting up and down. Store reconstituted Enzyme at -20°C and use within 2 months.

**Sample treatment:** liquid samples such as serum, plasma and fruit juices can be assayed directly. Because fruit juices may contain high concentrations of fructose, it is recommended to dilute juice sample 50-fold ( $n = 50$ ) in dH<sub>2</sub>O prior to assay. Milk samples should be cleared by mixing 600  $\mu$ L milk with 100  $\mu$ L 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300  $\mu$ L supernatant into a clean tube and neutralize with 50  $\mu$ L 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor  $n = 1.36$ ).

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. **Standards:** mix 12  $\mu$ L 20 mM Standard with 228  $\mu$ L dH<sub>2</sub>O (final 1000  $\mu$ M). Dilute standard in dH<sub>2</sub>O as follows.

No	1000 $\mu$ M STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Fructose ( $\mu$ M)
1	100 $\mu$ L + 0 $\mu$ L	100	1000
2	60 $\mu$ L + 40 $\mu$ L	100	600
3	30 $\mu$ L + 70 $\mu$ L	100	300
4	0 $\mu$ L + 100 $\mu$ L	100	0

Transfer 20  $\mu\text{L}$  diluted standards into separate wells of a clear flat-bottom 96-well plate.

*Samples:* transfer 20  $\mu\text{L}$  of each sample into separate wells of the plate.

3. *Color reaction.* Prepare enough Working Reagent by mixing, for each reaction well, 56  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Reconstituted Enzyme, 14  $\mu\text{L}$  PMS Solution and 14  $\mu\text{L}$  MTT Solution.

Keep Working Reagent protected from light. Add 80  $\mu\text{L}$  Working Reagent to each well. Tap plate to mix. *Do not expose Working Reagent to light for more than 5 minutes.* Incubate 60 min at room temperature in the dark.

4. Read optical density at 565nm (520-600nm).

*Note:* If the calculated fructose concentration of a sample is higher than 1000  $\mu\text{M}$ , dilute sample in water and repeat the assay. Multiply result by the dilution factor  $n$ .

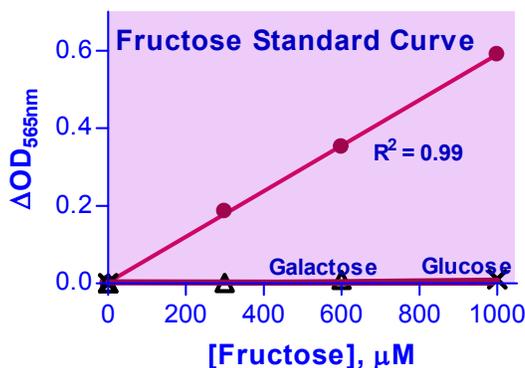
### CALCULATION

Subtract blank value (water, #4) from the standard values and plot the  $\Delta\text{OD}$  against standard concentrations. Determine the slope and calculate the fructose concentration of Sample,

$$[\text{Fructose}] = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{H}_2\text{O}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

$\text{OD}_{\text{SAMPLE}}$ ,  $\text{OD}_{\text{H}_2\text{O}}$  are optical density values of the sample and water.  $n$  is the dilution factor.

**Conversions:** 1 mM fructose equals 18 mg/dL, 0.018% or 180 ppm.



### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader.

### LITERATURE

1. Novelli G, Reichardt JK. (2000). Molecular basis of disorders of human fructose metabolism: past, present, and future. *Mol Genet Metab.* 71:62-65.
2. Pudek MR et al. (1990). Low concentration fructose determination in plasma adapted to the Cobas-Bio. *Clin Biochem.* 23:221-223.
3. Gabrielli m. (1978). serum fructose determination with centrifugal analyzers. *clin. chem.* 24:1990-1995.

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