Fructose Colorimetric/Fluorometric Assay Kit #BN00843

(Catalog #BN00843; 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 Assay Genie's Fructose Assay Kit, free fructose is enzymatically converted to β-glucose, which is then specifically converted to a product that reacts with GenieRed Probe to generate color (λ = 570nm) and fluorescence (Ex/Em = 535/587nm). The kit provides a rapid, simple, sensitive, and reliable method suitable for high throughput assayof D-fructose.

II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Fructose Assay Buffer	25 ml	WM	BN00843-1
GenieRed Probe (in DMSO)	200 µl	Red	BN00843-2A
Enzyme Mix	1 vial	Green	BN00843-4
Fructose Converting Enzyme	1 ml	Purple	BN00843-5
Fructose Standard (100mM)	100 µl	Yellow	BN00843-6

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: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at –20 °C, protect from light.

Fructose Converting Enzyme: (Enzyme is unstable when not in $(NH4)_2SO4$ Solution) Remove amount needed for assay (10 µl needed for each well); centrifuge x 5 min at top speed, carefully remove the supernatant and reconstitute with same volume Assay Buffer. Store rest at 4 °C. Use within 2 months after initial thaw.

Enzyme Mix: Dissolve in 220 µl 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 µl of Fructose Standard to 990 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/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 1.0 mM as in the colorimetric assay. Take 10 µl of the diluted Fructose Standard into 90 µl of Fructose Assay Buffer to make it 0.1 mM. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/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 min. Add samples up to 50 µl into 96 well plate. Bring the volume to 50 µl/well with Assay Buffer. 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 reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

36 µl Assay Buffer
2 µl GenieRed Probe**
2 µl Enzyme Mix
10 µl Fructose Converting Enzyme*

Mix well. Add 50 µl of the Reaction Mix to each well containing the Fructose Standard and test samples, mix well. Incubate the reaction for 2 hours 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 µl of the GenieRed probe can be used for each reaction to reduce the background florescence readings.

4. **Measurement:** Read OD 570 nm for colorimetric assay or Ex/Em = 535/587 nm for fluorometric assay in a microplate reader.

5. **Calculation:** 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:

$$C = S_a/S_v \quad \text{nmol/µl or mM}$$

Where: S_a is the sample amount of unknown (in nmol) from standard curve,
S_v is sample volume (µl) added to the wells.
Fructose Molecular Weight is 180.16 g/mol

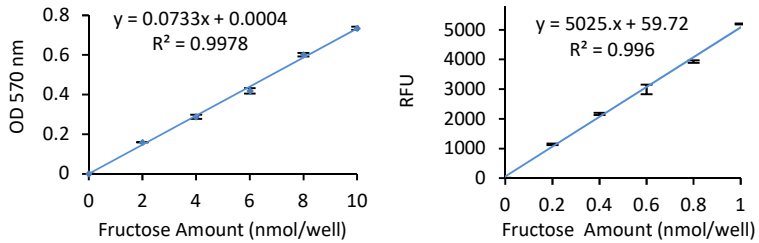

Figure: Fructose Standard Curve. Assays were performed follow the kit protocol.

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		