Product Information

Fructose Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2124-100 100 assays	Cap Color	Part Number
Fructose Assay Buffer	25 ml	WM	K2124-C-1
OxiRed Probe (in DMSO)	200 µl	Red	K2124-C-2
Enzyme Mix	1 vial	Green	K2124-C-3
Fructose Converting Enzyme	1 ml	Purple	K2124-C-4
Fructose Standard (100mM)	100 µl	Yellow	N1503

II. Introduction:

Fructose is a ketonic monosaccharide found in many plants and foods. Fructose, along with glucose and galactose, are three dietary monosaccharides that can be absorbed directly into the bloodstream during digestion. Fructose is the sweetest naturally occurring sugar.

The Fructose Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of fructose in cell or tissue culture supernatants based on colorimetric and fluorometric method. In the assay, free fructose is enzymatically converted to β-glucose, which is then specifically converted to a product that reacts with OxiRed Probe to yield fluorescence (Ex/Em = 535/587 nm) and color (λ = 570 nm). The kit is suited for high throughput assay of D-fructose.

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 $(NH4)_2SO_4$ 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:

Assay Buffer	36 µl
OxiRed Probe	2 µl
Enzyme Mix	2 µl
Fructose Converting Enzyme	10 µl

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 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 µl of the OxiRed 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 = Sa/Sv nmol/µl or mM

Where: Sa is the sample amount of unknown (in nmol) from standard curve,

Sv is sample volume (µl) added to the wells.

Fructose Molecular Weight is 180.16 g/mol.

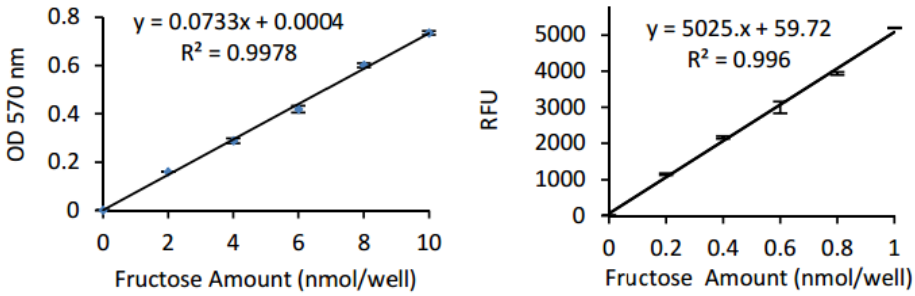

Figure: Fructose Standard Curve. Assays were performed follow the kit protocol.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates;

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

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com