Version 2 Last updated 10 August 2023

ab241022
Fructose Assay Kit (Fluorometric)

For the measurement of fructose in tissues, cells and biological fluids.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Fructose Assay Kit (Fluorometric) (ab241022) measures free fructose that is enzymatically processed with the formation of a metabolite which reacts with the Probe to generate fluorescence (Ex/Em = 535/587 nm). The kit provides a simple, highly sensitive, reliable method suitable for high throughput assay of D-fructose. Glucose interference can be removed by using the Sample Cleanup Mix. The kit can detect fructose in the range of 5 to 500 picomoles/well. This Kit does not work for serum or plasma.

Prepare Samples and Standards as directed.

Prepare Reaction Mix (samples and standards) and Sample Background Mix (sample controls). Add to samples, standards and controls, as appropriate

Incubate for 30 minutes at 37°C, protected from light.

Read fluorescence at Ex/Em = 535/587 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer V/Fructose Assay Buffer	25 mL	-20°C	-20°C
PicoProbe I/Probe	200 µL	-20°C	-20°C
Sample Cleanup Mix	1 vial	-20°C	-20°C
Converter Enzyme X/Conversion Enzyme	1 vial	-20°C	-20°C
Fructose Enzyme Mix	1 vial	-20°C	-20°C
Fructose Substrate Mix	1 vial	-20°C	4°C
Fructose Standard (100 mM)	100 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96 well white plate with flat bottom
- Dounce homogenizer (for cell/tissue samples)

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer V/Fructose Assay Buffer

Ready to use as supplied. Warm to room temperature before use.

5.2 PicoProbe I/Probe

Ready to use as supplied. Warm to > 20°C (to melt frozen DMSO) before use.

5.3 Sample Cleanup Mix

Dissolve with 220 µL Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

5.4 Converter Enzyme X/Conversion Enzyme

Dissolve with 220 µL Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

5.5 Fructose Enzyme Mix

Dissolve with 220 µL Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

5.6 Fructose Substrate Mix

Dissolve with 220 µL of Assay Buffer. Pipette up and down to dissolve. Stable for 2 months at 4°C.

5.7 Fructose Standard

Ready to use as supplied. Store at -20°C.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute the Fructose Standard to 1 nmol/µL by adding 10 µL of the 100 nmol/µL Standard to 990 µL of dH2O, mix well.
2. Dilute further to 10 µM by adding 10 µL to 990 µL of dH2O.
3. Add 0, 2, 4, 6, 8, 10 µL into a series of standards wells on a 96 well plate. Adjust volume to 50 µL/well with Assay Buffer to generate 0, 20, 40, 60, 80, 100 pmol/well of Fructose Standard.

ΔNote: Standard curves of more highly diluted fructose are possible if great care is taken while pipetting solutions.

Standard #	10 µM Fructose Standard (µL)	Assay Buffer V/Fructose Assay Buffer (µL)	Final volume standard in well (µL)	Fructose (pmol/well)
1	0	50	50	0
2	2	48	50	20
3	4	46	50	40
4	6	44	50	60
5	8	42	50	80
6	10	40	50	100

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Liquid samples:

Assay directly.

7.2 Cell or tissue samples:

1. Rapidly homogenize 10 - 100 mg tissue or 5 x 10^6 cells with 2 - 3 volumes of ice cold PBS or other buffer (pH ~8). Centrifuge at top speed for 10 minutes to remove insoluble materials.
2. Add 1 - 50 µL sample into duplicate wells of a 96-well plate and bring volume to 50 µL with Assay Buffer V/Fructose Assay Buffer.

ΔNote: Enzymes in sample may convert or consume fructose. We suggest deproteinizing samples using a perchloric acid/KOH protocol or 10 kDa molecular weight cut off spin filter to remove enzymes.

ΔNote: Samples may be homogenized in perchloric acid, then neutralized with 10 N KOH to minimize any loss of fructose. For tissues or cells containing low levels of free fructose, minimize sample dilutions as much as possible.

ΔNote: Some biological materials in samples (NADH, NADPH, etc.) will generate background readings. You may do a sample background control (omit Converter Enzyme X/Conversion Enzyme Mix from the reaction mix) to read the background then subtract the background from sample readings.

ΔNote: Samples such as and urine contain high amounts of glucose which will generate high background readings. Such samples need to be pretreated with 1 µL of the Sample Cleanup Mix for 30 minutes prior to analysis (dilution effect needs to be taken into consideration later, when calculating concentrations).

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction mix:

Prepare 50 µL of Reaction Mix and Sample Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer V/Fructose Assay Buffer	42	44
Converter Enzyme X/Conversion Enzyme	2	-
Fructose Enzyme Mix	2	2
Fructose Substrate mix	2	2
PicoProbe I/Probe ΔNote:	2	2

ΔNote: To minimize baseline fluorescence and self-quenching, the PicoProbe I/Probe addition should be based upon the standard curve range. For 0 - 500 pmol fructose use 2 µL/well and scale down proportionately.

8.2 Measurement:

1. Add 50 µl of the Reaction Mix to each well containing the Fructose Standards and Samples.
2. Add 50 µl of the sample background mix into Sample Background Control wells.
3. Incubate for 30 minutes at 37°C, protected from light.
4. Measure fluorescence at Ex/Em 535/587 nm.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each Standard, Control and Sample.
2. Correct background by subtracting the value of the 0 Fructose Standard from all Sample readings.
3. If significant, subtract the Sample Background Control from sample readings.
4. Plot the standard curve.
5. Apply the corrected sample readings to the standard curve to get the amount of Fructose in the sample wells.
6. Concentration of Fructose (µM) in the test samples is calculated as:

$$\text{Fructose concentration}(C) = \frac{Ay}{Sv} * D$$

Where:

C = Fructose concentration in test sample (pmol/µL = nmol/mL = µM).

Ay = amount of Fructose in the sample from the Standard Curve (pmol).

Sv= sample volume added to the sample well (µL).

10. Typical Data

Data provided **for demonstration purposes only.**

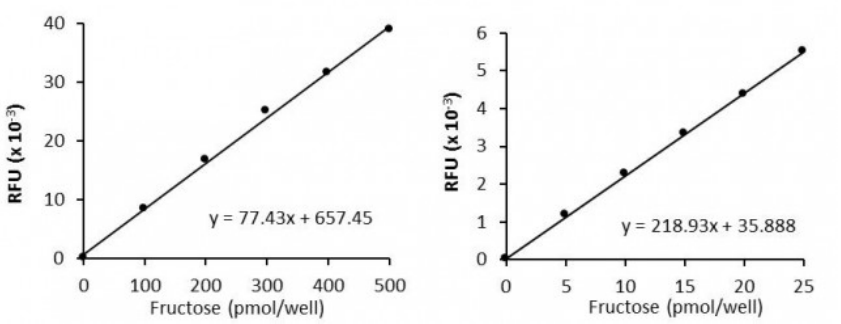

Figure 1. Fructose standard curves.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)