ab83380

Fructose Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of fructose levels in various samples.

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.

Version 4 Last Updated 23 August 2023

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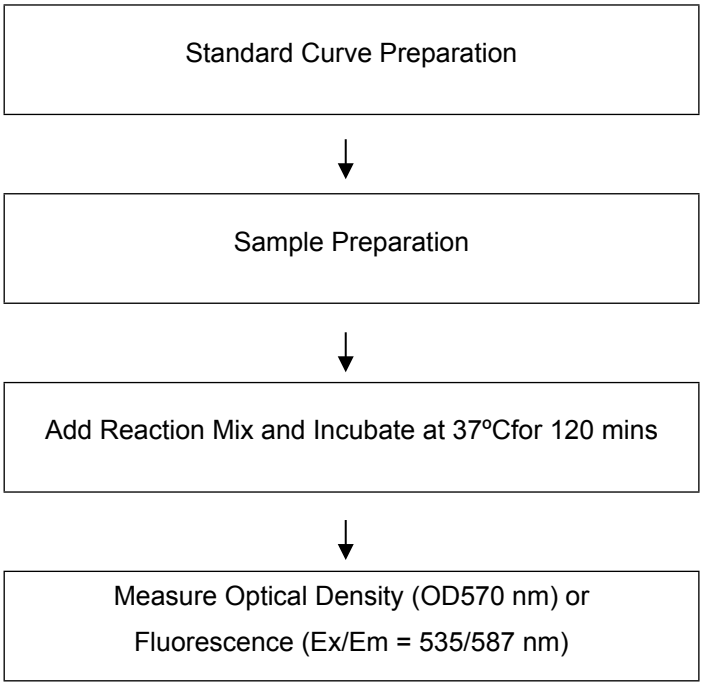
1. BACKGROUND

Fructose Assay Kit (colorimetric/fluorometric) (ab83380) converts free fructose enzymatically to β-glucose, which is then specifically converted to a product that reacts with OxiRed 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 assay of D-fructose.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Fructose Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe (in DMSO)	1 vial	-20°C	-20°C
Development Enzyme Mix II/Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Fructose Converter Mix/Fructose Converting Enzyme (in $(NH_4)_2SO_4$ Solution)	1 vial	-20°C	4ºC
Fructose Standard (100 mM)	100 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- 96 well plate: black plates (clear bottoms) for fluorometric assay; clear plates for colorimetric assay
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Fructose Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Fructose Standard:**

Ready to use as supplied. Store at -20°C. Keep on ice while in use.

9.3 **OxiRed Probe – in DMSO:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light. Once the probe is thawed, use with two months.

9.4 **Fructose Converter Mix/Fructose Converting Enzyme:**

Enzyme is unstable when not in $(NH_4)_2SO_4$ solution. Remove amount needed for assay (10 µL needed for each well); centrifuge for 5 min at top speed. Carefully remove the supernatant and reconstitute with same volume Assay Buffer. Store the remainder at +4 °C. Use within 2 months after initial thaw. Keep on ice while in use

9.5 **Development Enzyme Mix II/Enzyme Mix:**

Dissolve in 220 µL Assay Buffer. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20 °C. Use within two months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 1 mM fructose standard by diluting 5 µL of 100 mM Fructose Standard into 495 µL of Assay Buffer.

10.1.2 Using 1 mM fructose standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Fructose] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare 1 mM fructose standard by diluting 5 µL of 100 mM Fructose Standard into 495 µL of Assay Buffer

10.2.2 Prepare a 0.1 mM fructose standard by diluting 10 µL of 1 mM Fructose Standard in 90 µL of Assay Buffer.

10.2.3 Using 0.1 mM fructose standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Fructose] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. 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. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge 10 minutes at 4°C at 13,000 rpm using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 500 - 1,000 µL (or ~ 4 - 6 volumes) of Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 10 minutes at 4°C at 13,000 rpm using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer)
- (optional) Sample Background Control = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background wells = 50 µL Assay Buffer.

12.2 **Reaction Mix:**

Prepare Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	36	46
OxiRed Probe	2	2
Development Enzyme Mix II/Enzyme Mix	2	2
Fructose Converter Mix/Fructose Converting Enzyme	10	0

Component	Fluorometric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	37.6	47.6
OxiRed Probe*	0.4	0.4
Development Enzyme Mix II/Enzyme Mix	2	2
Fructose Converter Mix/Fructose Converting Enzyme	10	0

**NOTE:* *For fluorometric readings, using 0.4 µL/well of the probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.3 Add 50 µL of the appropriate Reaction Mix to each well.

12.4 Incubate at 37ºC for 2 hours protected from light.

12.5 Measure output on a microplate reader.

- Colorimetric assay: measure OD 570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the sample background from all standard and sample readings if applicable.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Fructose.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Sa = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of fructose in the test samples is calculated as:

$$Fructose\ Concentration = \left(\frac{Sa}{Sv}\right) * D$$

Where:

Sa = Amount of Fructose in the sample well (in nmol).

Sv = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Fructose Molecular Weight is 180.16 g/mol

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

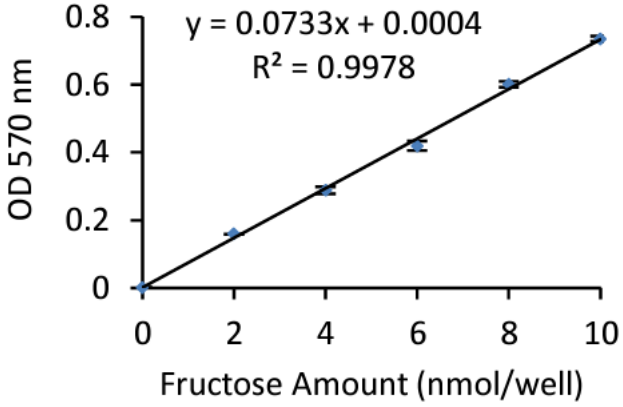


Figure 1: Typical fructose standard calibration curve using colorimetric reading.

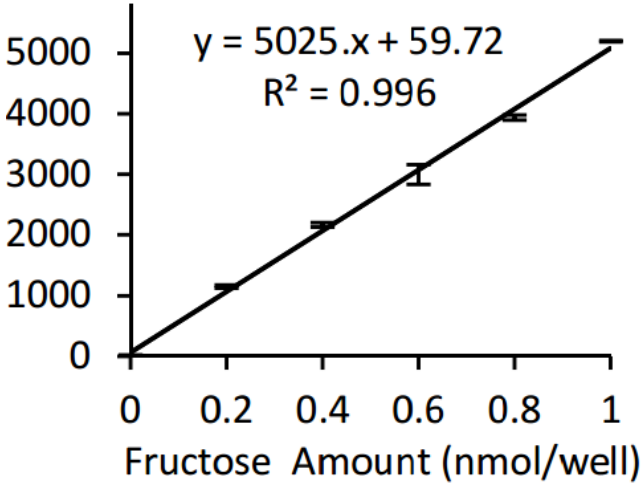


Figure 2: Typical fructose standard calibration curve using fluorometric reading.

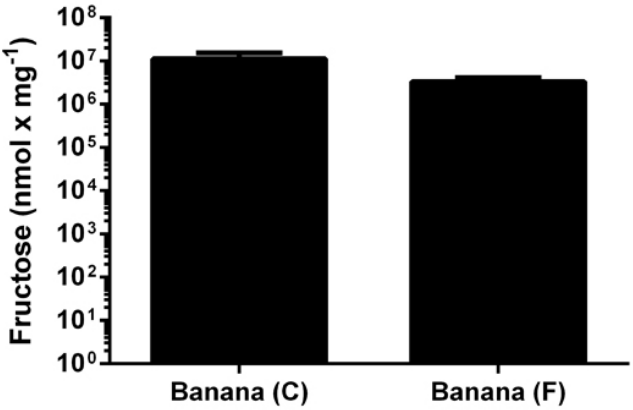


Figure 3. Fructose levels of banana, determined using the colorimetric (C) and the fluorometric (F) method (presented per mg of protein.

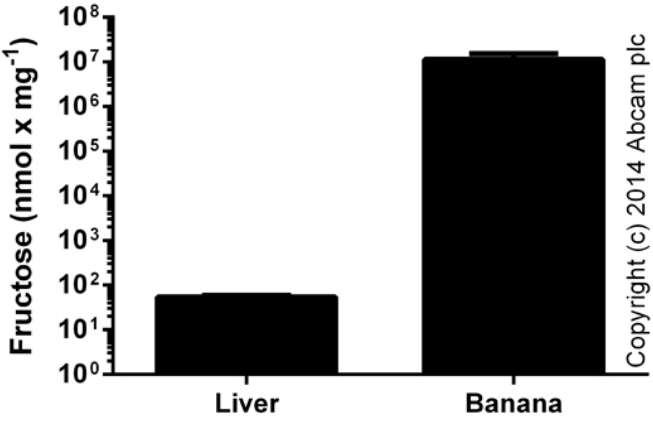


Figure 4: Fructose levels in mouse liver and banana, determined using the colorimetric method and presented per mg of protein.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, OxiRed probe and prepare Development Enzyme Mix II/enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Fructose Reaction Mix (Number samples + standards + 1)

Component	Colorimetric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	**36**	**46**
OxiRed Probe	**2**	**2**
Development Enzyme Mix II/Enzyme Mix	**2**	**2**
Fructose Converter Mix/Fructose Converting Enzyme	**10**	**0**

Component	Fluorometric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	**37.6**	**47.6**
OxiRed Probe	**0.4**	**0.4**
Development Enzyme Mix II/Enzyme Mix	**2**	**2**
Fructose Converter Mix/Fructose Converting Enzyme	**10**	**0**

- Add 50 µL Reaction Mix to wells.
- Incubate plate RT 30 mins.

- Measure plate at OD570nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Since the converting enzyme is unstable, will storing it at -20°C after use increase its shelf-life?

The converting enzyme is unstable when not in ammonium sulphate solution. It is recommended to store it at 4ºC after initial thaw and use it up within 2 months. Freezing and thawing even when stored in aliquots might decrease its activity dramatically. Hence storing it at -20ºC after first thaw is not recommended.

The glucose background in the samples is very high and the RFU measured is in 100,000 range. What is the best way to deal with this issue?

When doing the fluorometric assay it is important to use 0.4µL probe to reduce background. Also, the fluorometer sensitivity setting should be at "medium" or "low" so that noise does not get amplified.

Can this kit be used with samples from fruits?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources including fruits. Since the optimal conditions depend on the sample type, the protocol has to be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

Will alpha glucose interfere in this assay?

Alpha glucose typically makes up polymers like starch and should not interfere in this assay.

18. **INTERFERENCES**

19. **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)