Version 2 Last updated 6 September 2023

ab273329 Fructose-1,6-Bisphosphatase Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273329
(use https://www.abcam.cn/ab273329 for china, or
https://www.abcam.co.jp/ab273329 for Japan)

For the determination of Fructose-1,6-Bisphosphatase activity in adherent/suspension cells and tissue.

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-1,6-Bisphosphatase Activity Assay Kit (Colorimetric) (ab273329) provides a quick, reliable test that allows the measurement of Fructose-1,6-Bisphosphatase (FBP) enzymatic activity in various samples. In this assay, FBP hydrolyzes Fructose-1,6-Bisphosphate into Fructose-6-Phosphate (F6P). F6P is used in an enzyme coupled reaction which reduces a chromophore forming a product with a stable signal that can measured at OD = 450 nm.

The assay is simple, sensitive and can detect lower than 100 µU of FBP in variety of samples.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Measure absorbance in kinetic mode for 5-60 mins at 37°C.

Determine Fructose-1,6-Bisphosphatase activity using equation.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
F6P Standard /F6P Standard Lyophilized	1 vial	-20°C
Assay Buffer V/FBP Assay Buffer	25 ml	-20°C
Converter Enzyme X/FBP Converter Lyophilized	1 vial	-20°C
Developer IX/FBP Developer Lyophilized	1 vial	-20°C
FBP Positive Control Lyophilized	1 vial	-20°C
NADPH Substrate Mix/FBP Probe Lyophilized	1 vial	-20°C
FBP Substrate /FBP Substrate Lyophilized	1 vial	-20°C

7. Materials Required, Not Supplied

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

- 96-well plate with flat clear bottom
- Multi-well spectrophotometer (ELISA reader)
- 30% Glycerol
- Ammonium Sulfate Solution (Saturated, 4.1 M)

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- 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 all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

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

9.1 **Assay Buffer V/FBP Assay Buffer:**
Ready to use as supplied. Bring to room temperature before use.

9.2 **F6P Standard Lyophilized:**

Reconstitute with 100 µl dH_2O to generate 100 mM F6P Standard solution. Store at –20°C. Use within two months.

Keep on ice while in use.

9.3 **Converter Enzyme X/FBP Converter Lyophilized:**
Reconstitute the vial with 220 µl Assay Buffer V/FBP Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.

9.4 **Developer IX/FBP Developer Lyophilized:**
Reconstitute the vial with 220 µl Assay Buffer V/FBP Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.

9.5 **FBP Positive Control Lyophilized:**

Reconstitute with 50 µl of 30% Glycerol and mix thoroughly. Aliquot and store at –20°C. Use within two months.

9.6 **NADPH Substrate Mix/FBP Probe Lyophilized:**
Reconstitute with 600 µl Assay Buffer V/FBP Assay Buffer, mix well, aliquot and store at –20°C. Use within two months. Keep on ice while in use.

9.7 **FBP Substrate /FBP Substrate Lyophilized:**
Reconstitute the vial with 220 µl Assay Buffer V/FBP Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells ($5x10^6$) with 500 µl ice cold Assay Buffer V/FBP Assay Buffer, and place on ice for 10 mins. Centrifuge at 10,000 *X g* for 5 mins at 4°C and collect the supernatant.

10.2 Use ammonium sulfate to precipitate and remove small molecules that could interfere with the assay: Aliquot tissue samples (100 µl) into clean centrifuge tubes, add saturated 4.32 M ammonium sulfate to 65% saturation (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate) and place on ice for 30 mins. Spin down samples at 10,000 *x g* at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume with Assay Buffer V/FBP Assay Buffer.

10.3 Add 2-50 µl samples into a 96-well clear plate; adjust final volume to 50 µl with Assay Buffer V/FBP Assay Buffer.

10.4 For each sample add identical volume of 2-50 µl sample into two wells a 96-well clear plate – Sample [S] and background Control [BC] respectively; adjust the final volume to 50 µl with Assay Buffer V/FBP Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ Note: To control for sample background, prepare parallel sample wells as sample background controls

Δ Note: If you would like to determine specific GI activity in the samples use a BCA Protein Assay Kit - Reducing Agent Compatible or similar to determine protein concentration in samples.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute 100 mM F6P Standard to 1 mM F6P Standard by adding 10 µl of 100 mM F6P to 990 µl dH_2O.

11.2 Add 0, 2, 4, 6, 8 and 10 µl of 1 mM F6P Standard into a series of wells of a clear 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of F6P Standard.

11.3 Adjust volume to 50 µl/well with Assay Buffer V/Assay Buffer.

Standard #	1 mM F6P Standard (µL)	Assay Buffer V/FBP Assay Buffer (µL)	F6P (nmol/well)
1	0	50	0
2	2	48	2
3	4	46	4
4	6	44	6
5	8	42	8
6	10	40	10

12. Assay Procedure

– Keep on ice while in use.

12.1 **FBP Positive Control:** Dilute stock 1:1 with Assay Buffer V/FBP Assay Buffer (15 µl of FBP Positive Control + 15 µl Assay Buffer V/FBP Assay Buffer). Mix well. Add 1-20 µl of diluted Positive Control; adjust the final volume to 50 µl with Assay Buffer V/FBP assay buffer.

12.2 **Reaction Mix:**
Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Reaction mix:

	Reaction mix (µl)	Background Control mix (µl)
Assay Buffer V/FBP Assay Buffer	39	41
Converter Enzyme X/FBP Converter	2	2
Developer IX/FBP Developer	2	2
FBP Probe	5	5
FBP Substrate	2	---

12.3 Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples.
12.4 Add 50 µl of Background Control mix to each well containing the Background Control sample. Mix well.
12.5 Measure absorbance at OD = 450 nm in kinetic mode for 5-60mins at 37°C.

Δ Note: Incubation time depends on the Fructose-1,6-Bisphosphatase activity in samples. We recommend measuring absorbance in kinetic mode and choose two time points (t1 and t2) in the linear range to calculate the FBP activity of the samples. The F6P standard curve can be read in End-point mode (i.e., at the end of incubation time).

13. Calculations

13.1 Subtract the 0 standard reading from all standard readings.
13.2 Plot the F6P standard curve.
13.3 Correct sample background by subtracting the values derived from the sample background control from all sample readings.
13.4 Calculate the FBP activity of the test sample:

ΔOD = A2 – A1 measured at times t2 and t1 respectively.

13.5 Apply the ΔOD to the F6P standard curve to get B nmol of F6P generated by Fructose-1,6-Bisphosphatase during the reaction time (Δt = t2 - t1).

$$FBP\ activity\ = \frac{B}{(\Delta t\ x\ V)} X\ D = nmol/min/ml = mU/ml$$

B = F6P amount from the Standard Curve (nmol)
Δt = Reaction time ($t_2 – t_1$) (mins)
V = Sample volume added into the reaction well (ml)
D = Dilution factor (D=1 when sample is undiluted)

Unit Definition: One unit of Fructose-1,6-Bisphosphatase is the amount of enzyme that will generate 1.0 µmol of F6P per min at pH 8.0 at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

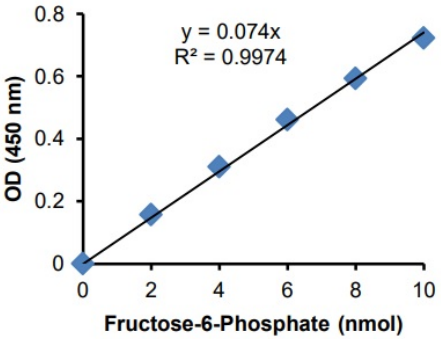

Figure 1. F6P standard curve.

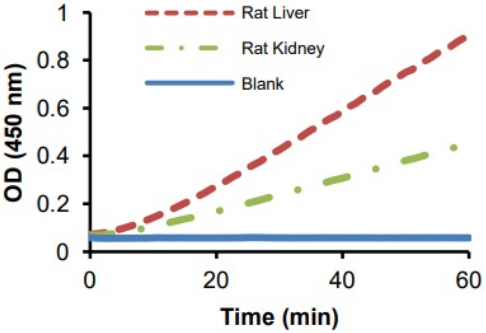

Figure 2. Kinetic measurement of Fructose-1,6-Biosphophatase activity from various samples.

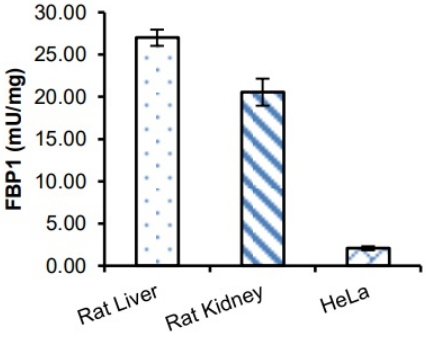

Figure 3. Specific FBP Activity was calculated in lysates prepared from rat liver (3.2 µg protein), rat kidney (8 µg protein) and HeLa lysate (4 µg protein).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)