CREATIVE BIOMART®
Assay Kit

Fructose-1,6-Bisphosphatase Colorimetric Activity Assay Kit

Product Information

Common Name

FBP

Cat.No. Kit-2530

Product Overview

FBP activity assay kit provides a quick, reliable test that allows the measurement of 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 enzymecoupled 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.

Description

Fructose-1,6-Bisphosphatase (FBP) (EC 3.1.3.11) is a rate-limiting enzyme for gluconeogenesis. It hydrolyzes Fructose-1,6-Bisphosphate releasing inorganic phosphate and Fructose-6-Phosphate that is further used in the synthesis of glucose. In humans, Fructose-1,6- Bisphosphatase can be found in the form of two isozymes: FBP1 and FBP2. FBP1 is mainly expressed in liver and kidneys, while FBP2 is expressed in all tissues. Deficiency of Fructose-1,6-Bisphoshatase causes hypoglycemia, metabolic acidosis, and unexpected death in infants. Recent studies have found elevated FBP levels subjects dealing with obesity and diabetes. Furthermore, inhibition of FBP increases glucose utilization and insulin sensitivity. Therefore, FBP could serve as a potential target for treating type II diabetes (insulin resistance).

Applications

- Measurement of Fructose-1,6-Bisphosphatase Activity in various tissues/cells.
- Analysis of gluconeogenesis pathway.
- Mechanistic studies of Molecular and/or Cell biology processes (diabetes, gluconeogenesis)

Storage

Store kit at -20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

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Size

100 assays

Kit Components

FBP Assay Buffer 25 ml
FBP Substrate 1 vial
FBP Converter 1 vial
FBP Developer 1 vial
FBP Probe 1 vial
FBP Converter 1 vial
FBP Substrate 1 vial
FBP Developer 1 vial

Materials Required but Not Supplied

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

Compatible Sample Types

- Animal tissues: Liver, Kidney etc.
- Cell culture: HeLa, Jurkat, etc.

Preparation

Reagent Preparation:
FBP Substrate, FBP Converter and FBP Developer: Reconstitute each vial with 220 µl FBP Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.
FBP Probe: Reconstitute with 600 µl FBP Assay Buffer, mix well, aliquot and store at -20°C. Use within two months. Keep on ice while in use.
F6P Standard: Reconstitute with 100 µl dH2O to generate 100 mM F6P Standard solution. Store at -20°C. Use within two months. Keep on ice while in use.

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FBP Positive Control: Reconstitute with 50 µl of 30% Glycerol and mix thoroughly. Aliquot and store at -20°C. Use within two months.

Assay Protocol

1. Sample Preparation: For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells (5x10^6) with 500 µl ice cold FBP Assay Buffer, and place on ice for 10 minutes. Centrifuge at 10,000 X g for 5 min at 4°C and collect the supernatant. 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 g at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume with FBP Assay Buffer. Add 2-50 µl samples into a 96-well clear plate; adjust final volume to 50 µl with FBP Assay Buffer. 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 FBP Assay Buffer. Positive Control: dilute stock 1:1 with FBP Assay Buffer (15 µl of FBP Positive Control + 15 µl FBP Assay Buffer). Mix well. Add 1-20 µl of diluted Positive Control; adjust the final volume to 50 µl with FBP assay buffer.
Notes:
a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
b. To control for sample background, prepare parallel sample wells as sample background controls.
c. If you would like to determine specific GI activity in the samples use BCA Protein Assay Kit.
2. F6P Standard Curve: Dilute 100 mM F6P Standard to 1 mM F6P Standard by adding 10 µl of 100 mM F6P to 990 µl dH2O. 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. Adjust volume to 50 µl/well with Assay Buffer.
3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

Reaction Mix	Background Control Mix
FBP Assay Buffer	39 µl	41 µl

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FBP Converter 2 µl 2 µl
FBP Developer 2 µl 2 µl
FBP Probe 5 µl 5 µl
FBP Substrate 2 µl -
Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples and 50 µl of Background Control mix to each well containing the Background Control sample. Mix well.
4. Measurement: Measure absorbance at OD = 450 nm in kinetic mode for 5-60 min at 37°C.
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 & 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).
5. Calculation: Subtract the 0 standard reading from all standard readings. Plot the F6P standard curve. Correct sample background by subtracting the values derived from the sample background control from all sample readings. Calculate the FBP activity of the test sample: ΔOD = A2 – A1 measured at times t2 and t1 respectively. 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).
Sample Fructose-1,6-Bisphosphatase Activity = B/(Δt X V) x Dilution Factor = nmol/min/ml/ = mU/ml
Where: B = the NADPH amount from standard curve (nmol).
ΔT = the reaction time (min).
V = the sample volume added into the reaction well (ml).
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.

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