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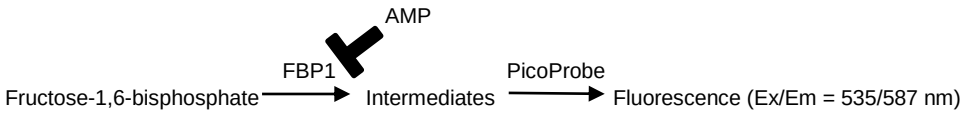
PicoProbe™ Fructose-1,6-Bisphosphatase Inhibitor Screening Kit (F)

rev 7/21

(Catalog # K399-100; 100 assays; Store at -20 °C)

I. Introduction:

Fructose-1,6-bispohphatase (FBP1) is responsible for the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate in the rate determining step of gluconeogenesis. FBP1 is a tetramer that plays a role in regulating adiposity and appetite. FBP1 has emerged as an important therapeutic target to treat diabetes type 2 to regulate glucose levels during gluconeogenesis. In **BioVision's FBP1 Inhibitor Screening Kit**, human FBP1 hydrolyzes fructose-1,6-bisphosphate to fructose-6-phosphate which oxidizes and reacts with a fluorogenic probe, that is detected at Ex/Em = 535/587 nm. The assay is fast, sensitive, high-throughput compatible and provides a quick and sensitive way for screening, studying and characterizing potential novel inhibitors of FBP1.



II. Application:

- Screening/studying/characterizing potential fructose-1,6-bisphosphatase inhibitors.

III. Kit Contents:

Components	K399-100	Cap Code	Part Number
FBP1 Assay Buffer	25 ml	WM	K399-100-1
FBP1 Substrate	1 vial	Clear	K399-100-2
Human FBP1	1 vial	Brown	K399-100-3
FBP1 Converter 1	1 vial	Green	K399-100-4
FBP1 Converter 2	1 vial	Purple	K399-100-5
FBP1 Developer	1 vial	Red	K399-100-6
PicoProbe™	0.4 ml	Blue	K399-100-7
AMP (Inhibitor)	1 vial	Yellow	K399-100-8

IV. User Supplied Reagents and Equipment:

- 96-well white plate with a flat bottom.
- Multi-well spectrophotometer (Fluorescent plate reader).

V. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **FBP1 Assay Buffer and PicoProbe™ (in DMSO)**: Bring to room temperature (RT) before use. Aliquot and store at -20 °C. Use within two months.
- **FBP1 Substrate:** Resuspend the vial in 440 µl of FBP1 Assay Buffer. Divide into aliquots and store at -20 °C. Use within two months.
- **Human FBP1:** Reconstitute the vial in 10 µl of distilled water and incubate for 20 min at RT. Divide into aliquots and store at -20 °C. Use within two months.
- **FBP1 Converter 1, FBP1 Converter 2, FBP1 Developer:** Reconstitute each vial in 220 µl of FBP1 Assay Buffer. Keep on ice until it completely dissolves. Aliquot the reconstituted FBP1 Converter 1, FBP1 Converter 2, and FBP1 Developer and store at -20 °C. Avoid repeated freeze thaw cycles. Use within two months.
- **AMP (Inhibitor):** Resuspend the vial in 100 µl of FBP1 Assay Buffer. Divide into aliquots and store at -20 °C. Use within two months.

VI. FBP1 Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control, Enzyme Control & Background Control Preparations:** Dissolve the candidate inhibitors into an appropriate solvent at 50x the concentration to be tested. Dilute the test inhibitor to 10x FBP1 Assay Buffer. **Sample Compound [S]**: Add 10 µl diluted test inhibitor (10X). **Enzyme Control [EC] (no inhibitor)**: add 10 µl Assay Buffer into desired wells. **AMP (inhibitor-IC):** Add 10 µl of AMP into desired well(s). **Background Control (BC)**: add 50 µl of FBP1 Assay Buffer into one of the wells. Bring the volume of S, EC, IC wells, to 40 µl using FBP1 Assay Buffer.

   Note: Solvents used to solubilize the inhibitors might affect FBP1 enzyme activity. If the solvent(s) is expected to affect the enzyme activity, prepare parallel well(s) for the Solvent Control [SC] to test the effect of the solvent on FBP1 activity by adding 40 µl of the final solvent concentration to the SC well(s). In case SC value is significantly different from EC, use its value to determine the effect of tested compound(s).

2. **Human FBP1:** Dilute the reconstituted Human FBP1 to 650-fold. For example, perform a serial dilution by taking 2 µl of the reconstituted Human FBP1 and add 50 µl of FBP1 assay buffer for a 26-fold dilution. Further dilute the enzyme by taking 2 µl of the 26X diluted enzyme and add it to 48 µl of FBP1 assay buffer for a 650-fold dilution. Prepare enough Human FBP1 enzyme for the number of samples to be tested. Add 10 µl of diluted Enzyme to the S, IC, EC, and SC wells. Incubate at 37 °C for 5 min.

3. Reaction Mix Preparation: Prepare 50 µl Reaction Mix for each well to be analyzed :

FBP1 Assay Buffer	36 µl
FBP1 Substrate	4 µl
FBP1 Converter 1	2 µl
FBP1 Converter 2	2 µl
FBP1 Developer	2 µl
PicoProbe™	4 µl

Add 50 µl of the Reaction Mix into all of the wells and mix well.

4. Measurement: Measure the fluorescent signal Ex/Em = 535/587 nm in kinetic mode at 37 °C for 15 min.

5. Calculation: Choose two time points t_1 & t_2, in the linear range of enzyme kinetics and obtain the corresponding ΔRFU (RFU_2 –RFU_1) during the reaction time ΔT (t_2 - t_1) for Enzyme Control ($\Delta RFU_{(EC)}$), and Test Inhibitor ($\Delta RFU_{(Test\ Inhibitor)}$). Use these values to obtain the percentage of inhibition.

Notes:

- Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S). If the data obtained from the solvent control(s) is significantly different from the EC, use this data instead of the EC data in the equation above.
- If Solvent control (SC) values are significantly different from the EC, use these values in the equation above after subtracting BC.

$$\textbf{Relative Activity}\ (\%) = \frac{(\textbf{Slope of EC} \quad - \quad \textbf{Slope of S})}{\textbf{Slope of EC}} \textbf{X 100}$$

$$\textbf{Relative Inhibition}\ (\%) = \frac{(\textbf{Slope of EC} - \textbf{Slope of S})}{\textbf{Slope of EC}}\ \textbf{X 100}$$

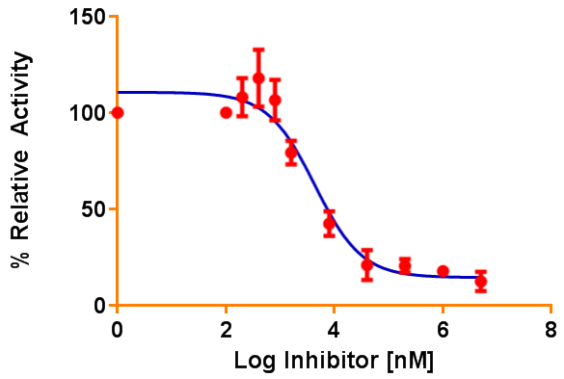


Figure: Inhibition of FBP1 activity by the control inhibitor, with an IC_{50} of 4 µM. Assay performed following kit protocol.

VII. Related Products:

PicoProbe™ Fructose Fluorometric Assay Kit (K611)
PicoProbe™ Fructose-6-Phosphate Fluorometric Assay Kit (K689)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)

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