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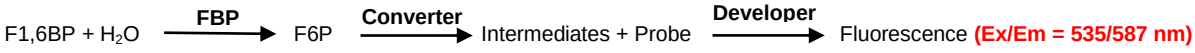
PicoProbe™ Fructose-1,6-Bisphosphate Assay Kit (F)

02/20

(Catalog # K2036-100; 100 assays; Store at -20°C)

I. Introduction:

Fructose-1,6-Bisphosphate (F1,6BP) is a fructose sugar and a key component of the glycolysis metabolic pathway. It is produced by the phosphorylation of Fructose 6-Phosphate (F6P) by phosphofructokinase (PFK). Additionally, F1,6BP is an allosteric activator of enzymes including PFK and pyruvate kinase. Furthermore, F1,6BP stimulates the Pentose Phosphate Pathway that produces glutathione and is associated with anti-convulsing effects in animal models. Recent studies have shown that F1,6BP can be taken up by various cell types thereby providing ATP in glycolysis without consuming ATP and preserving the organs such as heart, liver, kidney under ischemic conditions. **BioVision's Fructose-1,6-Bisphosphate Assay Kit** provides a quick and easy way to measure F1,6BP in a variety of sample types. In this Assay, F1,6BP is hydrolyzed by Fructose-1,6-Bisphosphatase (FBP) into inorganic phosphate and F6P. F6P then through several intermediate reactions reacts with the PicoProbe™ to generate a stable fluorophore measured at Ex/Em = 535/587 nm. The assay is simple, easy to perform, sensitive and can detect F1,6BP less than 0.5 µM in a variety of samples.



II. Applications:

- Measurement of F1,6BP in various tissues or cell lysates
- Analysis of Glycolysis Pathway and Pentose Phosphate Pathway

III. Sample Types:

- Animal tissues: Liver, Kidney etc.
- Plant tissues: Germinated Barley Seed, Spinach etc.

IV. Kit Contents:

Kit Components	K2036-100	Cap Code	Part Number
F1,6BP Assay Buffer	25 ml	WM	K2036-100-1
PicoProbe™	400 µl	Blue	K2036-100-2
F1,6BP Enzyme	1 vial	Orange	K2036-100-3
F1,6BP Converter A	1 vial	Purple	K2036-100-4
F1,6BP Converter B	1 vial	Green	K2036-100-5
F1,6BP Developer	1 vial	Red	K2036-100-6
F1,6BP Standard	4 vials	Yellow	K2036-100-7

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- 30% Glycerol
- 10 kDa Spin Column (BioVision Cat# 1997)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge all small vials prior to opening.

- **F1,6BP Assay Buffer:** Ready to use as supplied. Warm to room temperature (RT) before use. Store at 4°C after opening.
- **PicoProbe™ (in DMSO):** Ready to use as supplied. Warm to RT before use. Store at -20°C.
- **F1,6BP Enzyme:** Reconstitute with 220 µl of 30% Glycerol and mix thoroughly. Divide into aliquots and store at -20°C. Use within two months.
- **F1,6BP Converter A, F1,6BP Converter B and F1,6BP Developer:** Reconstitute each vial with 220 µl F1,6BP Assay Buffer. Pipette up and down to dissolve completely. Divide into aliquots and store at -20°C. Use within two months.
- **F1,6BP Standard:** Reconstitute each vial with 100 µl dH_2O to generate 10 mM F1,6BP stock Standard solution. Store at -20°C. Use within 2 weeks after reconstitution. Keep on ice while in use.

VII. Fructose-1,6-Bisphosphate Assay Protocol:

1. **Sample Preparation:** For whole cells or tissue lysate, rapidly homogenize tissue (10 mg) or cells (2 x 10^6) with 500 µl ice cold F1,6BP Assay Buffer and place on ice for 10 min. Centrifuge at 10,000 *x g* and 4°C for 10 min to remove any insoluble materials. Collect the supernatant to a new micro-centrifuge tube. Measure protein concentration in lysates with BCA Protein Assay Kit - Reducing Agent Compatible (BioVision Cat#K818) or similar. Use a 10 kDa Spin Column (BioVision Cat#1997) to remove any possible interfering enzymes and insoluble components. Use the flow through for the assay. For each Test Sample, add the same volume (2-40 µl) of Sample into three parallel wells of a white, flat bottom 96-well plate labeled as **Sample**, **Sample Background Control** and **Spiked Sample**.
2. **F1,6BP Standard Curve Preparation:** Dilute the reconstituted 10 mM F1,6BP stock Standard solution at 1:200 dilution by adding 5 µl of 10 mM F1,6BP stock Standard solution into 995 µl of F1,6BP Assay Buffer to generate 50 pmol/µl (50 µM) of F1,6BP Standard solution. Add 0, 2, 4, 6, 8 and 10 µl of 50 µM F1,6BP Standard solution into a series of wells in 96 well white plate to generate 0, 100, 200, 300, 400 and 500 pmol/well of F1,6BP Standard respectively. Adjust the volume of each well to 50 µl with F1,6BP Assay Buffer.

3. **Internal Spike Preparation:** Add 2 µl of 50 µM F1,6BP Standard solution (50 pmol/µl F1,6BP Standard) to the **Spiked Sample** well (100 pmol F1,6BP + Sample). The **Spiked Sample** well is used as an **Internal Standard** to correct for any Sample interference. Adjust the volume of all wells to 50 µl/well with F1,6BP Assay Buffer.
4. **Reaction Mix Preparation:** Prepare Reaction Mix (used for Sample and Standard wells) and Background Control Mix (used for Sample Background Control well) according to the table below. Make sufficient amount of each type of mix to add 50 µl to all assay wells of that type.

	Reaction Mix	Background Control Mix
F1,6BP Assay Buffer	38 µl	40 µl
F1,6BP Converter A	2 µl	2 µl
F1,6BP Converter B	2 µl	2 µl
F1,6BP Developer	2 µl	2 µl
PicoProbe™	4 µl	4 µl
F1,6BP Enzyme	2 µl	----

Mix well. Add 50 µl of Reaction Mix to each well containing the Standards, Spiked Samples and Samples. Add 50 µl of Background Control mix to Sample Background Control well(s) and use those values for Sample correction. Mix well. Incubate for 40 min at 37°C, protected from light.

5. **Measurement:** Incubate for 40 min at 37°C, protected from light. Measure the fluorescence of all wells at Ex/Em = 535/587 nm in end point mode.
6. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the F1,6BP Standard Curve. Subtract the Sample Background Control reading from its paired Sample reading to get the corrected Sample reading. Determine the F1,6BP amount in the Sample wells (X) based on the following equation:

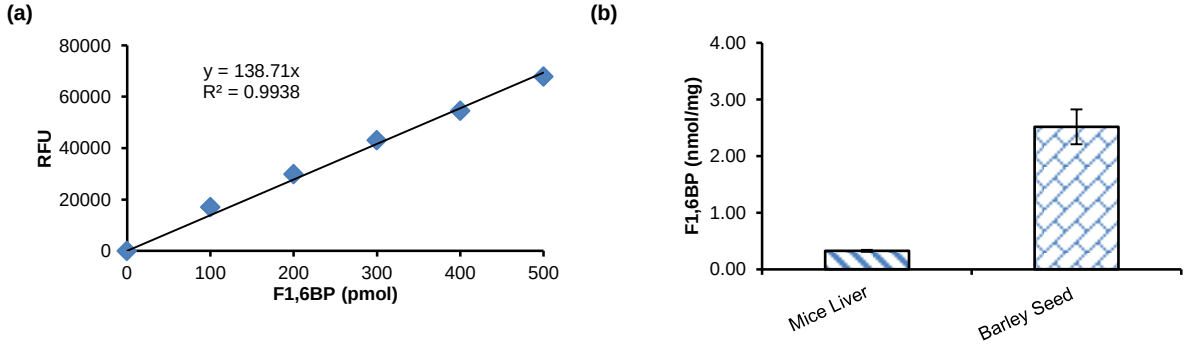
$$\text{Amount of F1,6BP amount in Sample well (X)} = \left(\frac{(\text{RFU}_{Sample\ (corrected)})}{(\text{RFU}_{(Spiked\ Sample\ )}-(\text{RFU}_{Sample})}\right) * 100 \text{ pmoles}$$

Sample F1,6BP concentration **= X/V x D = pmol/µl = nmol/ml = µmol/l or µM**

Where: **X** is the amount of F1,6BP from the calculation above (in pmol)
V is the sample volume added to the well (in µl)
D is the sample dilution factor (if applicable, D=1 for undiluted samples)
100 pmol is the amount of F1,6BP Standard spiked in the Spiked Sample well

Molecular weight of F1,6BP = 340.12

Sample F1,6BP concentration can also be expressed in pmol/µg or nmol/mg of Sample.



Figures: (a) F1,6BP Standard Curve generated using this kit. (b) Measurement of F1,6BP in mouse liver lysate (50 µg protein) and germinated barley seed lysate (G7) (10 µg protein). Assays were performed following the protocol.

VIII. Related Products:

PicoProbe™ F-6-P Assay Kit (K689)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
NAD/NADH Quantification Kit (K337)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Glucose and Sucrose Assay Kit (K616)
Fructose-1,6-Bisphosphatase Activity Assay Kit (K590)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Fructose Assay kit (K619)
Hexokinase Assay Kit (K789)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)

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