ab284537 – Fructose-1,6-Bisphosphate Assay Kit (Fluorometric)

For the measuring of Fructose-1,6-Bisphosphate (F1,6BP) in animal or plant tissues.
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 5	25 mL	-20°C
Converter Mix G	1 vial	-20°C
Developer Mix G	1 vial	-20°C
Developer Mix P	1 vial	-20°C
F1,6BP Enzyme	1 vial	-20°C
F1,6BP Standard	4 vials	-20°C
PicoProbe I	0.4 mL	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and F1,6BP Assay Buffer, Converter Mix G as Converter Enzyme X and F1,6BP Converter A, Developer Mix G as Development Enzyme Mix IX and F1,6BP Converter B, and Developer Mix P as Developer IX and F1,6BP Developer. The kit mechanism has not changed.

Materials Required, Not Supplied

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- 30% Glycerol
- 10 kDa Spin Column
- dH2O

Reagent Preparation

– Before using the kit, spin the tubes prior to opening. Components are stable for at least three months.

Assay Buffer 5: Ready to use as supplied. Warm to room temperature (RT) before use. Store at 4°C after opening

PicoProbe I: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

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.

Converter Mix G, Developer Mix G and Developer Mix P: Reconstitute each vial with 220 µL Assay Buffer 5. 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 Standard stock solution. Store at -20°C. Use within 2 weeks after reconstitution. Keep on ice while in use.

Version 4b, Last updated March 11, 2026

Assay Protocol

Sample preparation:

1. For whole cells or tissue lysate, rapidly homogenize tissue (10 mg) or cells (2 x 10^6) with 500 µL ice cold Assay Buffer 5 and place on ice for 10 min.
2. 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.
3. Measure protein concentration in lysates with BCA Protein Assay Kit Reducing Agent Compatible (ab207003) or similar.
4. Use a 10 kDa Spin Column to remove any possible interfering enzymes and insoluble components.
5. Use the flow through for the assay.
6. 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.

F1,6BP Standard Curve Generation (Optional)

1. Dilute the reconstituted 10 mM F1,6BP stock Standard solution at 1:200 dilution by adding 5 µL of 10 mM F1,6BP Standard stock solution into 995 µL of Assay Buffer 5 to generate 50 pmol/µL (50 µM) of F1,6BP Standard solution.
2. 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.
3. Adjust the volume of each well to 50 µL with Assay Buffer 5.

Internal Spike Preparation

1. 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).
2. The Spiked Sample well is used as an Internal Standard to correct for any Sample interference.
3. Adjust the volume of all wells to 50 µL/well with Assay Buffer 5

Reaction Mix Preparation

1. Prepare Reaction Mix (used for Sample and Standard wells) and Background Control Mix (used for Sample Background Control well) according to the table below.
2. Make sufficient amount of each type of mix to add 50 µL to all assay wells of that type.

	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 5	38	40
Converter Mix G	2	2
Developer Mix G	2	2
Developer Mix P	2	2
PicoProbe I	4	4
F1,6BP Enzyme	2	-

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

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.

Calculation

1. If you performed the optional standard curve, subtract the 0 Standard reading from all Standard readings.
    a. Plot the F1,6BP Standard Curve and use this as a reference.

ΔNOTE: the internal spike method is the most accurate way to determine F1,6BP amount in a well, interpolating from the standard curve will not account for matrix effects.

2. Subtract the Sample Background Control reading from its paired Sample reading to get the corrected Sample reading ($RFU_{corrected}$) .
3. To Determine the F1,6BP amount in the Sample wells (X) using the internal spike, use the following equations:

   Amount of F1,6BP Sample well (X) = $\left(\frac{(RFU\ (corrected))}{(RFU\ (spiked\ sample)) - (RFU\ (sample))}\right) * 100$ pmoles

4. Sample concentration can be calculated as follows:

Sample F1,6BP concentration = $\frac{X}{V} * D = \mathbf{pmol/\mu l} = \mathbf{nmol/ml} = \mu\mathbf{mol/l\ or\ \mu M}$

Where:

X = the amount of F1,6BPusing the internal spike (in pmol)
V = the sample volume added to the well (in µL)
D = the sample dilution factor (if applicable, D=1 for undiluted sample)
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.

Commented [AJ1]: @Schmitt, Kyle C please heck these calculations. We have the customer make a standard curve but didn't tell them how to use it. Do we need to add a note about when a spike is appropriate? Is it indeed optional?

Commented [AJ2R1]: @Schmitt, Kyle C fixed it. Please check.

Technical Support

For all technical or commercial enquiries please go to:

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