

**Amplite® Colorimetric Fructose-6-Phosphate Activity Assay Kit**

 Catalog number: 11330  
 Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11330)
Component A: F6P Probe	Freeze (< -15 °C), Minimize light exposure	1 Vial (1 mL)
Component B: F6P Assay Buffer	Freeze (< -15 °C)	1 Bottle
Component C: F6P Enzyme Mix	Freeze (< -15 °C)	1 Bottle
Component D: F6P Developer	Freeze (< -15 °C)	1 Bottle
Component E: ATP	Freeze (< -15 °C), Minimize light exposure	1 Bottle (4 mL)
Component F: Fructose-6-Phosphate (F6P) Standard	Freeze (< -15 °C)	1 Vial

**OVERVIEW**

The Amplite® Colorimetric Fructose-6-Phosphate Assay Kit is a highly sensitive and simple method of quantifying F6P present in a variety of samples. This kit uses a coupled enzyme assay where F6P, in the presence of ATP, is converted to fructose-1,6-diphosphate and ADP by the PFK enzyme. The aldolase enzyme then converts fructose-1,6-diphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Subsequently, glyceraldehyde-3-phosphate dehydrogenase, in the presence of NAD, converts glyceraldehyde-3-phosphate into glyceraldehyde-1,3-bisphosphate and NADH. The NADH produced then reduces a colorless substrate to a colored product (A450 nm), which is proportional to the fructose-6-phosphate present. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute. Fructose-6-phosphate (F6P) is an important intermediate generated during glycolysis. It is produced by the isomerization of glucose-6-phosphate by phosphoglucose isomerase. It serves as a precursor to fructose 1,6-bisphosphate. Beyond its role in energy production, F6P can also be shunted to the non-oxidative pentose phosphate pathway as a means of generating pentose phosphates for nucleotide production. F6P levels surge in rapidly proliferating cells like cancer cells, making it a potential metabolic biomarker in diseases.

**AT A GLANCE**
**Important Note**

Thaw all the kit components at room temperature before starting the experiment.

**Protocol Summary**

1. Prepare the test samples, and the serially diluted F69 standards (50 μL).
2. Add the F6P working solution (50 μL).
3. Incubate for 10-30 minutes at room temperature.
4. Measure the absorbance at 450 nm.

**KEY PARAMETERS**
**Absorbance microplate reader**

Absorbance	450 nm
Recommended plate	Clear bottom

**PREPARATION OF STOCK SOLUTIONS**

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

**Fructose-6-Phosphate (F6P) Standard**

1. Add 330 μL of PBS buffer to the vial containing the Fructose-6-Phosphate (F6P) standard (Component F) to prepare a 20 mM (50 nmol/μL) F6P stock solution.

**Note:** Store the solution at -20°C. Avoid repeated freeze/thaw cycles.

**ATP Stock Solution**

1. Add 100 μL of ddH<sub>2</sub>O to the vial of ATP (Component E) to create a 100X ATP stock solution.

**Note:** Store this solution at -20°C and avoid repeated freeze/thaw cycles.

**F6P Developer Stock Solution**

1. Add 100 μL of ddH<sub>2</sub>O to the vial of F6P Developer (Component D) to create a 100X F6P Developer stock solution.

**Note:** Store this solution at -20°C and avoid repeated freeze/thaw cycles.

**PREPARATION OF STANDARD SOLUTIONS**

For convenience, use the Serial Dilution Planner:  
<https://www.aatbio.com/tools/serial-dilution/11330>

**F6P Standard**

Add 5 μL of the 20 mM F6P standard solution to 495 μL of PBS Buffer to create a 200 μM F6P solution, STD7. Next, take 150 μL of the STD7 solution and perform a 1:2 serial dilution in PBS Buffer to generate a series of diluted F6P standards (STD7 to STD1).

**PREPARATION OF WORKING SOLUTION**
**F6P Working Solution**

1. Add 1.0 mL of F6P Probe (Component A) to 4 mL F6P Assay Buffer (Component B), and mix well.
2. Transfer the 5 mL buffer mixture from above to the F6P Enzyme Mix bottle, and mix well.
3. Add 50 μL of the ATP stock solution to the same bottle, and mix well.
4. Add 50 μL of the F6P Developer stock solution to the same bottle, and mix well.

**Note:** Prepare the F6P working solution fresh before each experiment and protect it from light. A 5 mL preparation is

sufficient for 100 tests. Adjust the volume proportionally based on the number of tests you plan to conduct.

**Note:** Alternatively, one can prepare a 50X stock solution of F6P Enzyme Mix by adding 100 µL of ddH<sub>2</sub>O to the bottle of F6P Enzyme Mix (Component C) and mix thoroughly to dissolve the enzyme completely. Next, to prepare the F6P working solution, combine the 50X stock solution with the other components listed in the 'F6P Working Solution' section above, following the specified proportions.

### SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of F6P standards and test samples in a 96-well solid black microplate. (STD = F6P Standards (STD1-STD7, 3.125-200 µM), BL = Blank Control, TS = Test Samples)

BL	BL	TS	TS
STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

**Table 2.** Reagent composition for each well.

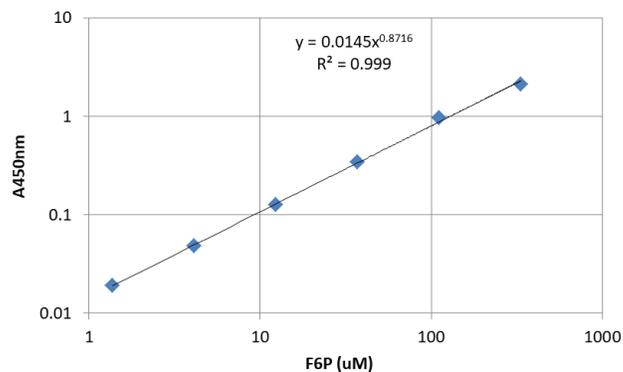
Well	Volume	Reagent
STD 1- STD 7	50 µL	Serial Dilutions (3.125 - 200 µM)
BL	50 µL	PBS
TS	50 µL	Test Sample

1. Prepare the F6P standards (STD1-7), blank controls (BL), and test samples (TS) as outlined in Tables 1 and 2. When using a 384-well plate, add 25 µL of reagent to each well instead of the standard 50 µL.
2. Add 50 µL of F6P Working Solution to each well containing the F6P standard, blank control, and test samples. If you are using a 384-well plate, add 25 µL of F6P Working Solution to each well instead.
3. Incubate at room temperature for 10-30 minutes, protected from light.
4. Monitor the absorbance intensity with an absorbance microplate reader at 450 nm.

### EXAMPLE DATA ANALYSIS AND FIGURES

The absorbance reading from the blank wells (containing only PBS) is used as a control and subtracted from the readings of wells containing F6P standards and test samples. Figure 1 shows the standard curve for F6P. To determine the F6P concentrations in your samples using this standard curve, we recommend using the Online Linear Regression Calculator available at:

<https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator>



**Figure 1.** The dose response of F6P was measured using the Amplitude® Colorimetric Fructose-6-Phosphate Assay Kit. The assay was conducted on a 96-well clear bottom microplate, with incubation for 30 minutes, and readings were taken at 450 nm using a ClarioStar microplate reader (BMG).

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