Amplite® Colorimetric Phosphofructokinase (PFK) Activity Assay Kit

Catalog number: 11329
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11329)
Component A: PFK Probe	Freeze (< -15 °C)	1 Vial (1 mL)
Component B: PFK Assay Buffer	Freeze (< -15 °C)	1 Bottle (4 mL)
Component C: PFK Enzyme Mix	Freeze (< -15 °C)	1 Bottle
Component D: PFK Developer	Freeze (< -15 °C)	1 Vial
Component E: ATP	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component F: NADH Standard	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component G: PFK Positive Control	Freeze (< -15 °C)	1 Vial

OVERVIEW

The Amplite® Colorimetric Phosphofructokinase (PFK) Activity Assay Kit provides a simple and direct procedure for precise measurement of PFK enzyme activity in various biological samples. This kit is based on a coupled enzyme assay, in which fructose-6-phosphate in the presence of ATP is converted to fructose1,6-diphosphate and ADP by PFK enzyme. This ADP is then converted to AMP and NADH by an enzyme mix. The resulting NADH reduces a colorless substrate to a colored product which absorbs at 450 nm and is proportional to the PFK activity present. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. Phosphofructokinase (PFK) is an enzyme of glycolysis pathway catalyzing the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. This is a crucial step of glycolysis as this reaction determines the overall rate of glycolysis. PFK enzyme activity is regulated by multiple cofactors and post-translational modifications. Deficiencies in PFK activity are linked to a rare genetic disorder known as glycogen storage disease type VII (GSD-VII), also known as Tarui disease. Additionally, deficiencies in PFK activity may also contribute to exercise-induced myopathy and rhabdomyolysis in certain individuals.

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 NADH standards (50 µL).
2. Add the PFK 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

NADH Standard

1. Add 200 µL of PBS buffer to the vial containing the NADH standard (Component F) to prepare a 2 mM (2 nmol/µL) NADH stock solution.

   Note: Store the solution at -80°C. Avoid repeated freeze/thaw cycles.

PFK Positive Control Stock Solution

1. Reconstitute the Phosphofructokinase (PFK) Positive Control (Component G) by adding 100 µL of ddH2O, and mix well by pipetting.

   Note: Store at -20°C, and use within 2 months of reconstitution.

ATP Stock Solution

1. Add 100 µL of ddH2O 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.

PFK Developer Stock Solution

1. Add 100 µL of ddH2O to the vial of PFK Developer (Component D) to create a 100X PFK Developer stock solution.

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

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11329

NADH Standard

Add 30 µL of a 2 mM standard solution to 270 µL of PBS Buffer to create a 200 µM NADH solution, labeled as 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 NADH standards (STD7 to STD1).

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PREPARATION OF WORKING SOLUTION

PFK Working Solution

1. Add 1.0 mL of PFK Probe (Component A) to 4 mL PFK Assay Buffer (Component B), and mix well.

2. Transfer the 5 mL buffer mixture from above to the PFK 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 PFK Developer stock solution to the same bottle, and mix well.

   Note: Prepare the PFK 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 PFK Enzyme Mix by adding 100 µL of ddH2O to the bottle of PFK Enzyme Mix (Component C) and mix thoroughly to dissolve the enzyme completely. Next, to prepare the PFK working solution, combine the 50X stock solution with the other components listed in the 'PFK Working Solution' section above, following the specified proportions.

SAMPLE EXPERIMENTAL PROTOCOL

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

BL	BL	PFK Positive Control	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
PFK Positive Control	50 µL	PFK Positive Control
TS	50 µL	Test Sample

1. Prepare the NADH standards (STD1-7), blank controls (BL), PFK Positive Control, 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 PFK Working Solution to each well containing the NADH standard, blank control, PFK Positive Control, and test samples. If you are using a 384-well plate, add 25 µL of PFK 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 NADH standards, PFK positive samples, and test samples. Figure 1 shows the standard curve for NADH. To determine the NADH 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

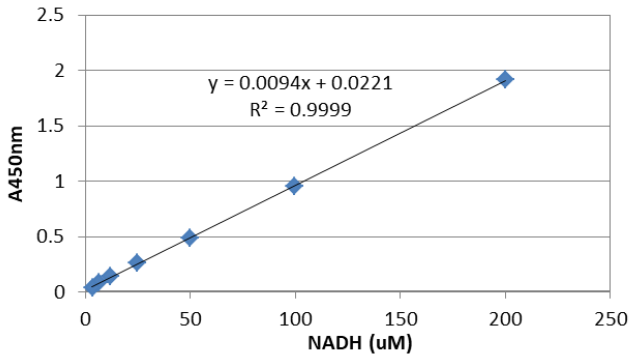

Figure 1. NADH dose response was measured using the Amplite® Colorimetric Phosphofructokinase (PFK) Assay Kit. The measurements were taken on a 96-well clear bottom microplate after a 30-minute incubation period using a ClarioStar microplate reader (BMG) at 450 nm.

Data Analysis Example

Calculate PFK Activity Example

- PFK positive control: 500X dilution in PBS
- 30min absorbance readings: 1.019
- Control: 0.382

Calculate the NADH generated during 30 minutes:

PFK-Positive Control	BG	PFK BG Corrected Signal	NADH (µM) in 30 min	Activity (mU/mL= µM/min)
1.019	0.382	0.637	65.4	2.2

Note:

- One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute
- nmole/min/mL=µM/min=mU/mL

DISCLAIMER

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