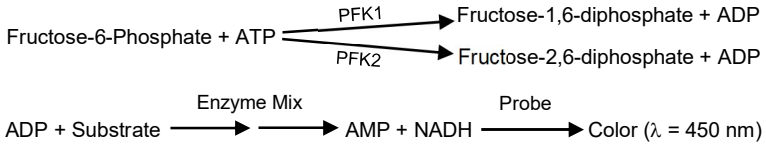
Phosphofructokinase (PFK) Activity Colorimetric Assay Kit

(Catalog # BN00989; 100 assays; Store at -20°C)

I. Introduction:

Phosphofructokinase (PFK) is a key glycolytic enzyme and plays a major regulatory role during glycolysis. This enzyme is present in bacteria, plants and animals. There are 2 types of PFKs - PFK1 and PFK2. In the presence of ATP, PFK1 & PFK2 catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate and fructose-2,6-diphosphate respectively and ADP. PFK has 3 major isoforms in mammals: PFK-M (muscle), PFK-L (liver) and PFK-P (platelet). In humans, PFK deficiency causes glycogen storage disease, also called Tarui's disease, which is characterized by exercise-induced muscle weakness and cramps. On the other hand, increased PFK activity contributes to cancer cell proliferation and tumorigenicity. Early detection of abnormal phosphofructokinase activity is crucial for diagnosis, prediction and therapeutic strategy. In Assay Genie's Phosphofructokinase Activity Assay kit, PFK converts fructose-6-phosphate and ATP to fructose-diphosphate and ADP. The ADP in the presence of substrate and enzyme mix is converted to AMP and NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. PFK activity assay is simple, robust, and rapid and can detect phosphofructokinase activity less than 1 mU.



II. Application:

- Measurement of phosphofructokinase activity in various tissues/cells.
- Analysis of glucose metabolism and cell signaling in various cell types.
- Screening anti-cancer drugs.

III. Sample Type:

- Animal tissues: Liver, Brain, Heart, Muscles etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	BN00989	Cap Code	Part Number
PFK Assay Buffer	27 ml	WM	BN00989-1
PFK Substrate (lyophilized)	1 vial	Blue	BN00989-2
ATP (lyophilized)	1 vial	Orange	BN00989-3
PFK Enzyme Mix (Lyophilized)	1 vial	Green	BN00989-4
PFK Developer (Lyophilized)	1 vial	Red	BN00989-5
NADH Standard (Lyophilized)	1 vial	Yellow	BN00989-6
Positive Control (Lyophilized)	1 vial	Purple	BN00989-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **PFK Substrate:** Reconstitute with 220 µl Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.
- **ATP:** Reconstitute with 220 µl dH_2O. Store at -20°C. Use within two months. Keep on ice while in use.
- **PFK Enzyme Mix:** Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- **PFK Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 40 µl Assay Buffer to generate 10 mM NADH Standard stock solution. Store at –20°C. Use within two months. Keep on ice while in use.
- **Positive Control:** Reconstitute with 100 µl Assay Buffer and mix thoroughly. Aliquot and store at –20°C.

VIII. Phosphofructokinase Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (20 mg) or cells (2 x 10^6) with 200 µl ice cold Assay Buffer on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (100 µg) per well. Adjust final volume to 50 µl with Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from ADP and NADH in the sample.
   Note: a) Small molecules in some samples such as liver may interfere with PFK Activity Assay. ADP, NADH and other interfering small molecules can be removed by 10 KD spin column. Specifically, pre-wet the spin column with dH_2O, spin down at 10000 x g for 2 min,

remove the dH_2O from upper and bottom reservoirs. Add 100 µl test sample, spin down at 10000 x g for 10 min at 4°C, collect the samples from the upper reservoirs and bring the volume to 100 µl with assay buffer. Discard the filtrate.
b) For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **NADH Standard Curve:** Dilute NADH Standard 10 fold by adding 10 µl NADH Standard stock solution to 90 µl Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of 1 mM diluted NADH Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of NADH Standard. Adjust final volume to 50 µl/well with Assay Buffer.
3. **Positive Control:** Add 10-20 µl of diluted PFK Positive Control into well(s). Adjust final volume to 50 µl with Assay Buffer.
4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
PFK Assay Buffer	42 µl	44 µl
PFK Enzyme Mix	2 µl	2 µl
PFK Developer	2 µl	2 µl
ATP	2 µl	2 µl
PFK Substrate	2 µl	----

Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples and 50 µl of Background Control Mix to each well containing the sample background control. Mix well.

5. **Measurement:** Incubate for 20-60 min at 37°C and measure OD_{450nm}.
Note: Incubation time depends on the Phosphofructokinase activity in the samples. We recommend measuring OD in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the PFK activity of the samples. The NADH standard curve can read in Endpoint mode (i.e., at the end of incubation time).
6. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Calculate the Phosphofructokinase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Phosphofructokinase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Phosphofructokinase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml = mU/ml

Where: **B** is the NADH amount from Standard Curve (nmol).
ΔT is the reaction time (min).
V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of phosphofructokinase is the amount of enzyme that generates 1.0 µmol of NADH per min at pH 7.4 at 37°C.

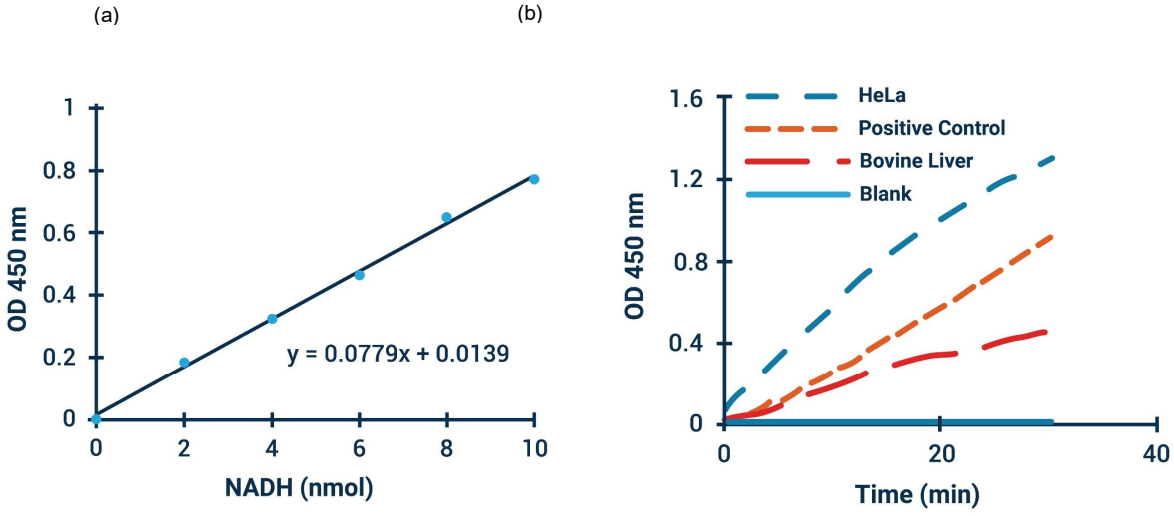


Figure 1: NADH standard curve (a). Phosphofructokinase activity in bovine liver and HeLa cell lysate (b). Assays were performed following kit protocol.

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